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Imaging techniques to study drug transporter function in vivo

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Imaging techniques to study drug transporter function in vivo

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Abstract

Transporter systems involved in the permeation of drugs and solutes across biological membranes are recognized as key determinants of pharmacokinetics. Typically, the action of membrane transporters on drug exposure to tissues in living organisms is inferred from invasive procedures, which cannot be applied in humans. In recent years, imaging methods have greatly progressed in terms of instruments, synthesis of novel imaging probes as well as tools for data analysis. Imaging allows pharmacokinetic parameters in different tissues and organs to be obtained in a non-invasive or minimally invasive way. The aim of this overview is to summarize the current status in the field of molecular imaging of drug transporters. The overview is focused on human studies, both for the characterization of transport systems for imaging agents as well as for the determination of drug pharmacokinetics, and makes reference to animal studies where necessary. We conclude that despite certain methodological limitations, imaging has a great potential to study transporters at work in humans and that imaging will become an important tool, not only in drug development but also in medicine. Imaging allows the mechanistic aspects of transport proteins to be studied, as well as elucidating the influence of genetic background, pathophysiological states and drug-drug interactions on the function of transporters involved in the disposition of drugs.

List of Abbreviations

ABC, ATP-binding cassette ; AD, Alzheimer's disease ; ADME, Absorption, Distribution, Metabolism and Elimination ; ATP, adenosine triphosphate ; *AUC*, area under the curve ; BBB, blood-brain barrier ; BCRP, breast cancer resistance protein ; BP_{ND} , non-displaceable binding potential ; *CL*, total clearance ; CT, computed tomography ; CYP, cytochrome P450 ; DDI, drug-drug interaction ; K_1 , influx rate constant ; k_2 , efflux rate constant ; MAG3, mercaptoacetyltriglycine ; MATE, multidrug and toxin extrusion protein ; MDR, multidrug resistance ; MRI, magnetic resonance imaging ; MRP, multidrug resistance-associated protein ; OAT, organic anion transporter ; OATP, organic anion-transporting polypeptide ; OCT, organic cation transporter ;

OCTN, organic cation / carnitine transporter ; P-gp, P-glycoprotein ; PBPK, physiologically based pharmacokinetic ; PET, positron emission tomography ; PK, pharmacokinetics ; SLC, solute carrier ; SNP, single nucleotide polymorphism ; SPECT, single photon emission computed tomography ; TKI, tyrosine kinase inhibitor ; V_T , total volume of distribution.

Keywords

Pharmacokinetics, molecular imaging, membrane transporters, positron emission tomography, magnetic resonance imaging, single photon emission computed tomography

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1. Introduction

The pharmacologic effect of drugs is directly dependent on the local concentration of the drug at its respective target and hence critically depends on the pharmacokinetics (PK) of the drug (Fung & Jusko, 2015; Levy, 1966). PK includes determining the absorption, distribution, metabolism and excretion (ADME) of a drug or xenobiotic, and describes the concentration-time profile, typically determined in the circulation (Fan & de Lannoy, 2014). It is evident that sampling drugs in blood plasma does not give direct and accurate information on the concentration of a drug at its target, which very often is located in tissues, outside of the circulation. Current clinical pharmacokinetic studies do not provide information on the penetration of drugs into tissues or across barriers, e.g. the blood-brain barrier (BBB), placenta or tumor barriers, to name a few.

ADME of drugs and xenobiotics is governed by many factors. Crossing of plasma membranes or other biological membranes by drugs and other solutes requires in general proteins, i.e. transport systems (Kell, 2015). (Drug) transporters can be divided very generally into systems mediating the uptake of substances into cells and systems mediating the efflux of substances from cells (Hediger et al., 2013). Uptake of solutes into cells is largely mediated by members of the solute carrier (SLC) gene series (Hediger et al., 2013). Export may be mediated by SLC transporters or, if against steep concentration gradients, by members of the group of adenosine triphosphate (ATP)-binding cassette (ABC) transporters. Once in a cell, many drugs and solutes are metabolized. Metabolism will affect both the total and intracellular concentration of transporter substrates. The interplay of transport into cells and intracellular metabolism generates a dilemma in the elucidation of molecular mechanisms involved in drug metabolism and elimination (Benet, 2009).

Current methodology for measuring drug concentrations in tissues requires invasive procedures (Chu et al., 2013). In such situations, imaging methods come into play, as they allow molecules in the body to be visualized in a non-invasive manner and even more so as they allow detailed information to be obtained on time-dependent changes in drug concentrations in organs or tissues.

In parallel to the acceptance of transporters as the key protein systems allowing the crossing of solutes over biological membranes (Giacomini et al., 2010; Hillgren et al., 2013; Suzuki & Sugiyama, 2000; van Montfoort et al., 2003), the role of drug transporters in imaging is increasingly being appreciated (Kannan et al., 2009; Kilbourn, 2017; Kusuvara, 2013; Langer, 2016; Mann et al., 2016; Marie et al., 2017; Stieger et al., 2012; Testa et al., 2015; Van Beers et al., 2012). Non-invasive imaging methods have therefore been developed to reveal and quantify drug transporter function in clearance and non-clearance organs, as a prerequisite to the local pharmacologic/toxicological effect, metabolism and elimination. The aim of this review is to provide an overview of the application of imaging methods dedicated to the study of drug transporter function in vivo.

2. Imaging methods for in vivo pharmacokinetics

2.1 In vivo imaging modalities

Current research in the field of translational imaging of drug transporters is predominantly based on clinically feasible imaging approaches. This includes nuclear imaging such as single photon emission computed tomography (SPECT) or positron emission tomography (PET) as well as magnetic resonance imaging (MRI). All of these approaches rely on the use of drug transporter substrates, which are detected using appropriate imaging modalities. The main advantage of these imaging techniques is their non-invasive or minimally-invasive nature. This allows a repetitive study of the same living subject, including humans, in identical or comparative conditions at different time points, thus harnessing the statistical power of longitudinal studies and reducing the number of individuals required (Willmann et al., 2008). This contrasts with the more conventional destructive biodistribution approaches, in which animals are sacrificed at predefined time points. In this framework, molecular imaging benefits from suitable temporal resolution to address the impact of carrier-mediated transport systems on the kinetics of drug disposition in vivo (Cunha et al., 2014).

Scintigraphy captures emitted radiation from administered gamma-emitting radioisotopes to create two-dimensional and poorly quantitative “planar” acquisitions. Tomographic acquisitions and computed tomography reconstruction (i.e. SPECT) aim at overcoming this limitation by providing 3D images of the distribution of the signal. Usually one, two or three detectors are slowly rotated around the body, leading to a considerable loss in time-resolution (Frey et al., 2012). SPECT has traditionally been considered as a non-quantitative imaging method, but recent advances in medical physics have now made SPECT a quantitative method due to coupling with a computed tomography (CT) scan in hybrid SPECT/CT scanners. Quantification with clinical SPECT/CT scanners, however, remains of slightly lower performance than with PET, especially in small volume regions, due to marginally poorer image spatial resolution (typically ~7-10 mm for SPECT versus ~5-8 mm for PET) (Bailey & Willowson, 2013). SPECT benefits from good sensitivity for the detection of radioactivity, allowing for the use of imaging agents at tracer doses with a limited risk of toxicity. Despite its technical limitations, SPECT represents a relevant and cost-effective tool for the study of transporter function in a conventional hospital environment (Fig. 1) (Willmann et al., 2008).

PET uses radioactive isotopes emitting positrons (β^+ decay), which collide with electrons in the tissue. Subsequent annihilation of these particles releases two photons, which can be detected and allow calculation of the point where the positron and the electron collided. Today, numerous PET tracers are used in medical practice (Vallabhajosula et al., 2011). PET imaging has also been used for decades in drug development (Willmann et al., 2008). The advantages of PET over SPECT include higher sensitivity (detection efficiency) and better temporal and spatial resolution. PET imaging benefits from straightforward 3D quantifiable recordings. Owing to the high sensitivity of PET scanners, radiopharmaceutical agents used for PET imaging can be administered at tracer doses, usually in the range of a few micrograms (Fig. 1). PET is a functional imaging technique which only gives limited anatomical information. For the delineation of organ substructures on PET images, anatomical information is often required. This can be obtained either by performing

additional scans on stand-alone MRI or CT scanners and co-registration of the data from the two imaging modalities, or by using integrated imaging systems (PET/CT, PET/MRI), which provide both functional and anatomical information in a single scanning session (Beyer et al., 2000, 2017).

Compared with nuclear imaging techniques, MRI is a non-irradiant imaging modality. MRI measures the energy released in the form of a radiofrequency of atoms, which realign to a strong magnetic field after being forced out of this alignment, and requires atomic nuclei with an odd number of protons and/or neutrons. MRI produces pictures of anatomy and of physiological processes with high spatial resolution and excellent soft tissue contrast. Several MRI contrast agents are now available, including paramagnetic gadolinium compounds (Xiao et al., 2016). Compared with nuclear imaging, which can be performed using tracer doses of radiopharmaceutical agents, the contrast agents used for MRI must be administered at pharmacologic doses in order to be detected. The sensitivity is thus limited (micro- to millimolar) and quantification of the absolute concentration of the contrast agent is challenging (Sinharay & Pagel, 2016). MRI is nonetheless able to capture the rapid temporal signal changes (contrast enhancement) associated with the presence of a contrast agent in tissues. MRI thus offers semi-quantitative data derived from high temporal and spatial resolution images that are of interest for studying transporter function in vivo (Georgiou et al., 2017) (Fig. 1).

2.2 Chemistry of imaging probes

To date, transporter imaging probes are usually not molecules designed de novo, but are mostly derived from known drug molecules, drug metabolites or from endogenous compounds (e.g. bile acids) (Kusuhara, 2013; Mairinger et al., 2011; Testa et al., 2015). The classical positron-emitting radionuclides for PET imaging are carbon-11 (^{11}C , half-life: 20.4 min) and fluorine-18 (^{18}F , half-life: 109.7 min). These radionuclides are produced in a cyclotron, and the synthesis of PET radiotracers is typically performed immediately before administration in dedicated lead-shielded automated synthesis modules. Today, the number of specialized PET centers is limited, which limits the applicability of this technique (Willmann et al., 2008) (Fig. 1).

^{11}C is the preferred PET radionuclide as it allows radiolabeling of drugs without structural modifications. Due to the versatility of PET radiochemistry, PET imaging benefits from a large portfolio of available radiotracers when compared with other clinical imaging modalities (Fig. 1). ^{11}C -labeling is most commonly performed by ^{11}C -methylation of phenolic hydroxyl, carboxylic acid or amino groups (Fig. 2). Recent alternative approaches to ^{11}C -labeling have been developed, thus giving the possibility of labeling a wider range of chemical structures with ^{11}C (Li & Conti, 2010). For drugs which contain fluorine, ^{18}F may be employed for radiolabeling (Wuest, 2007). The latter offers the advantage of a longer radioactive half-life than that of ^{11}C , thereby enabling the measurement of drug tissue PK over several hours (Brunner et al., 2004). A typical ^{18}F -labeling reaction is the nucleophilic substitution reaction with [^{18}F]fluoride of suitable leaving groups (Fig. 2). PET radiochemistry is usually performed at high molar activity (molar activity = ratio of radioactivity to mass, given for instance as GBq/ μmol). Therefore, the chemical mass of unlabeled drug contained in a typically injected amount of a PET tracer (approximately 400 MBq) is very low (< 100 μg). This satisfies the definition of a microdose set forth by regulatory authorities, which requires less extensive preclinical toxicology testing for applications in humans than is required for standard phase one clinical trials, thereby facilitating first-in-human studies (ICH Expert Working Group, 2009).

For SPECT radiochemistry, typical radionuclides for the radiolabeling of small molecules are technetium-99m ($^{99\text{m}}\text{Tc}$, half-life, 6.1 h) and iodine-123 (^{123}I , half-life: 13.3 h). $^{99\text{m}}\text{Tc}$ allows for clinical grade and kit-based preparation of radiopharmaceutical agents for human use and often provides labels with suitable metabolic stability in vivo. Several $^{99\text{m}}\text{Tc}$ -labeled imaging agents used in the clinics have been re-purposed as probes for molecular imaging of drug transporters (Kusuhara, 2013).

The variety of available contrast agents for MRI is very limited. MRI contrast agents predominantly contain metals (mostly the transition metal gadolinium) associated by complex chemistry. MRI is increasingly considered as a molecular imaging method owing to the availability of contrast agents

to detect molecular processes at the tissue level (Sinharay & Pagel, 2016). Gadoxetate (gadolinium-ethoxybenzyl-diethylenetriamine-pentaacetic acid, Gd-EOB-DTPA, Fig. 2) is a MRI contrast agent which induces liver-specific contrast enhancement due to transporter-mediated uptake into hepatocytes and biliary secretion of gadoxetate (van Montfoort et al., 1999). This contrast agent benefits from a suitable complex stability in vivo without any apparent biotransformation, making it suitable to study liver transporter function in vivo.

2.3 Modeling transporter function in vivo

Of the available imaging techniques, PET is the preferred modality for kinetic modeling purposes as it provides fully quantitative tissue concentrations of radioactivity (Fig. 1). For dynamic contrast-enhanced MRI, conversion of contrast enhancement in tissue into absolute concentration levels of contrast agent is feasible but challenging (Ingrisch & Sourbron, 2013; Sourbron & Buckley, 2013). When the time course of radiotracer in arterial blood (the so-called arterial input function) is also known, compartmental modeling approaches can be employed to derive pharmacokinetic parameters describing the tissue distribution of radiotracer. The modeling approaches used for analysis of imaging data are conceptually similar to modeling approaches used in the pharmaceutical sciences, but they employ different terminology (Innis et al., 2007). The gold standard to measure the arterial input function is arterial blood sampling. Pharmacokinetic modeling of imaging data can, for instance, provide the exchange rate constants of radiotracer between plasma and tissue compartments as outcome parameters (Innis et al., 2007). As ABC and SLC transporters are localized at blood-tissue interfaces, these exchange rate constants have been identified as the key parameters reflecting transporter activities. For example, the activity of efflux transporters at the BBB, such as P-glycoprotein (P-gp, *ABCB1*) or breast cancer resistance protein (BCRP, *ABCG2*), was shown to mainly affect the transfer rate constant of radiotracer from plasma into brain (K_1). A reduction in efflux transporter activity at the BBB (e.g. by administration of a transporter inhibitor) was shown to increase the K_1 value (Muzi et al., 2009; Wagner et al., 2009). Conceptually, efflux transporters at the BBB should also affect the efflux rate constant from

brain to plasma (k_2) (Syvänen et al., 2006), which was indeed observed for certain compounds (Pottier et al., 2016). Inhibition of an uptake transporter in the sinusoidal membrane of hepatocytes will lead to a reduction in the transfer rate constant or in the uptake clearance from blood into liver ($CL_{\text{uptake,liver}}$), which may be determined with graphical analysis approaches (integration plot analysis) (Takashima et al., 2012). Integration plot analysis has also been used to obtain intrinsic efflux clearances of radiotracers from liver into bile ($CL_{\text{int,bile}}$) or from kidney into urine ($CL_{\text{int,urine}}$) to measure the activities of efflux transporters located at the canalicular membrane of hepatocytes or at the brush border membrane of kidney proximal tubule cells, respectively (Shingaki et al., 2015; Takashima et al., 2013; Traxl et al., 2017).

Depending on the organ investigated, modeling approaches can become very complex. For instance, the modeling of liver data is complicated by the fact that the liver receives dual blood supply, via the hepatic artery (~ 25%) and via the portal vein (~ 75%). Portal blood cannot be directly sampled in humans. Therefore, modeling approaches either have to rely on image-derived portal blood curves, which may be prone to imaging artefacts due to the small size of the investigated structures, or on data acquired in preclinical species (e.g. pigs) in which portal blood can be sampled (Ørntoft et al., 2017; Sørensen et al., 2016). Figure 3 shows a kinetic model used for quantification of hepatic disposition of the radiolabeled bile acid [^{11}C]cholylsarcosine in humans (Ørntoft et al., 2017). Similar kinetic modeling approaches to those used for the analysis of PET data have also been applied to SPECT data (Pfeifer et al., 2013; Neyt et al., 2013) and to MRI data (Sourbron et al., 2012; Saito et al., 2013).

2.4 Metabolism of imaging probes

Like most xenobiotics, imaging probes may often undergo significant *in vivo* biotransformation. Paradoxically, “molecular imaging” techniques are devoid of “molecular resolution”. Nuclear imaging techniques (PET, SPECT) can only measure total radioactivity in tissues. Radioactivity originating from radiolabeled parent drug cannot be distinguished from radioactivity originating from radiolabeled metabolites (Pike, 2009). While metal chelates used as probes to study drug

transporters with SPECT and MRI often benefit from good in vivo stability, ^{11}C - and ^{18}F -labeled small, drug-like molecules used in PET studies often undergo extensive metabolism (Pike, 2009). The study of drug transporters with radiotracers which are extensively metabolized can provide misleading results, in that changes in radiotracer metabolism can be erroneously interpreted as changes in transporter activity. If circulating radiolabeled metabolites are present in blood, total radioactivity counts in blood samples can be corrected for these radiolabeled metabolites using ex vivo chromatographic methods. On the other hand, radiolabeled metabolites in tissues cannot be corrected for and will inevitably contribute to the imaging signal. During radiotracer development, tissue-penetrant radiometabolites can be detected and identified in tissue lysates obtained from terminal experiments in rodents (Pike, 2009). However, species differences in radiotracer metabolism may lead to the presence of tissue-penetrant radiolabeled metabolites in humans where not observed in rodents, or vice versa. The contribution of radiolabeled metabolites to the tissue PET signal in humans can only be indirectly inferred, for instance by the observation that pharmacokinetic modeling yields results which are inconsistent with the presence of a single radioactive species. The best approach to assess the contribution of radiolabeled metabolites to the PET signal in humans would be to directly radiolabel the metabolite of interest for PET imaging experiments, which is, however, very labor intensive and therefore rarely done. However, according to the authors' own experience, PET tracers based on known drug molecules which are mainly excreted in the form of metabolites may undergo a relatively low degree of metabolism over the short duration of an imaging experiment (e.g. 60-90 min for ^{11}C radiotracers), so that they may still be useful to study transporter function. One such example is erlotinib, for which mass balance studies in humans indicated that only < 2% of total radioactivity excreted into feces was composed of unmetabolized parent compound (Ling et al., 2006). PET experiments with ^{11}C erlotinib in mice, on the other hand, indicated that at 25 min after radiotracer injection the majority of radioactivity in plasma and different tissues and fluids was in the form of unmetabolized ^{11}C erlotinib (Traxl et al., 2015), suggesting that this radiotracer can be used to study transporter

function. One approach to overcome the limitation of radiotracer metabolism may be the radiolabeling of drug metabolites and their use as transporter imaging probes with better metabolic stability, as exemplified by the P-gp imaging probe [^{11}C]*N*-desmethyl-loperamide (Lazarova et al., 2008) or by the BCRP imaging probe [^{11}C]SC-62807 (Takashima et al., 2013). Moreover, the problem of radiolabeled metabolites can be overcome by performing data analysis over a short and early time window after radiotracer injection in which radiotracer metabolism is negligible. For instance, the P-gp probe substrate [^{11}C]verapamil is extensively metabolized in vivo, generating radiolabeled metabolites which are also P-gp substrates and which are taken up from periphery into the brain (Luurtsema et al., 2005). Some investigators have therefore used data from the first few minutes after radiotracer injection only, when [^{11}C]verapamil accounts for more than 80% of the plasma radioactivity, to determine K_1 as a parameter of P-gp function at the BBB (Ikoma et al., 2006) (Muzi et al., 2009). Similarly, integration plot analysis to determine the uptake clearance of radiotracers from blood into the liver usually relies exclusively on data from the first few minutes after radiotracer injection, where the contribution of radiolabeled metabolites can often be neglected (Kaneko et al., 2018). For the analysis of apical ABC efflux transporters in the liver or kidney, on the other hand, good metabolic stability of the PET tracer is mandatory as metabolism may otherwise confound the determination of $CL_{\text{int,bile}}$ or $CL_{\text{int,urine}}$ values.

3 Imaging probes to study transporter function

3.1 Liver transporters

The liver is a key organ involved in the metabolism and excretion of xenobiotics. Consequently, the expression of a large variety of transporters has been observed in this organ and the role of some of these transporters is well established in pharmacokinetic drug-drug interactions (DDIs) and in adverse drug actions in the liver. Organic anion-transporting polypeptides (OATPs) have a very broad substrate specificity and are key transporters for drugs entering hepatocytes (Hagenbuch & Stieger, 2013). In addition, OATPs are known to mediate the transmembrane

transport of a considerable number of imaging agents (Marie et al., 2017). The founding members of the *SLCO* gene superfamily encoding OATPs were shown to transport the MRI contrast agent gadoxetate (van Montfoort et al., 1999) (Fig. 2). The SPECT tracer [^{99m}Tc]mebrofenin (Fig. 2) is likewise taken up into the liver via OATPs and can be used for hepatobiliary imaging (de Graaf et al., 2011; Ghibellini et al., 2008). However, transport of most hepatic imaging agents is not restricted to OATPs but may also include organic anion transporters (OATs), organic cation transporters (OCTs) and ABC transporters (Kilbourn, 2017; Mairinger et al., 2011; Stieger et al., 2014; Testa et al., 2015).

The transporters for bile salts in hepatocytes have been identified and characterized at the molecular level (Hagenbuch & Stieger, 2013; Stieger, 2011). Bile salt derivatives are excellent target molecules for developing imaging agents to study liver transporters of importance in PK. For example, cholylsarcosine is a synthetic bile acid which is resistant to deconjugation and dehydroxylation (Schmassmann et al., 1990). A ¹¹C-labeled version of this bile acid was successfully used to visualize in vivo the kinetics of bile acid secretion in pigs (Frisch et al., 2012; Sørensen et al., 2016). In a recent proof-of-concept study, this bile acid was found to be a suitable probe to non-invasively assess bile flow in humans with liver disease (Ørntoft et al., 2017). In addition, the successful synthesis of ¹¹C-labeled derivatives of taurocholic acid, taurochenodeoxycholic acid, taurodeoxycholic acid, tauroursodeoxycholic acid and tauroolithocholic acid was reported (Schacht et al., 2016). All derivatives display comparable biodistribution in pigs, and enterohepatic circulation of the taurocholate derivative could be observed. Using a different synthetic strategy, an ¹⁸F-labeled chenodeoxycholic derivative was developed and demonstrated by PET imaging to be concentrated in the liver of mice in a time-dependent manner (Jia et al., 2014b). However, as the click chemistry-based ¹⁸F-labeling reaction led to modification of the carboxyl group, some concerns were raised as to whether this tracer was handled by the same hepatic transporters as endogenous bile acids (Frisch & Sørensen, 2014). Recently, an ¹⁸F-labeled derivative of unconjugated cholic acid (3β-[¹⁸F]fluorocholic acid, Fig. 2)

was shown to be a substrate of Na⁺-taurocholate co-transporting polypeptide (NTCP, *SLC10A1*), OATP1B1 (*SLCO1B1*) and OATP1B3 (*SLCO1B3*) (De Lombaerde et al., 2017), all of which are known to be involved in hepatocellular bile acid and bile salt uptake (Stieger, 2011). Furthermore, this bile acid derivative inhibited bile salt export pump (BSEP, *ABCB11*)-mediated taurocholate and MRP2-mediated estradiol-17 β -glucuronide transport. In mice, pretreatment with rifampicin or bosentan (Fattinger et al., 2000, 2001; Stieger et al., 2000) was shown to interfere with hepatocellular transport of 3 β -[¹⁸F]fluorocholic acid (De Lombaerde et al., 2017).

In addition to studying the function of bile acid transporters, PET has been used to study ABC efflux transporter function in the canalicular membrane of hepatocytes. For instance, the radiolabeled metabolite of the COX-2 inhibitor celecoxib [¹¹C]SC-62807 was shown to undergo biliary secretion mediated by mBCRP and should thus allow measurement of BCRP function in the liver (Takashima et al., 2013). PET imaging with [¹¹C]dehydropravastatin allows assessment of the transport activities of OATPs and MRP2 (*ABCC2*) in the rat and human liver (Kaneko et al., 2018; Shingaki et al., 2013).

MRI is one of the methods used to examine patients for presence of focal liver lesions (Pastor et al., 2014). MRI contrast agents are typically negatively charged (Xiao et al., 2016) and are substrates of transport systems for organic anions (Marie et al., 2017). Gadoxetate was found to be transported by human OATPB1 and OATP1B3, but not by OATP2B1 (*SLCO2B1*) (Leonhardt et al., 2010). In addition, gadoxetate is a weak substrate of NTCP, which may further contribute to its liver uptake (Leonhardt et al., 2010). Gadoxetate was also shown to be transported by MRP2 (Jia et al., 2014a), which mediates its biliary secretion in rats (Jia et al., 2014a). In one study, gadoxetate was found not to be transported by OATP1A2 (*SLCO1A2*) (van Montfoort et al., 1999), while others identified gadoxetate as a substrate of OATP1A2 and MRP3 but not of the apical sodium-dependent bile acid transporter (ABST, *SLC10A2*) and OCT3 (*SLC22A3*) (Jia et al., 2014a). Lagadec and coworkers recently reported a correlation between the hepatic extraction fraction of gadoxetate and the expression of rOATP1A1 in a rat model of advanced liver fibrosis

(Lagadec et al., 2015). BOPTA (gadolinium benzyl-oxypropionictetraacetate) is another MRI contrast agent labeled with gadolinium. In situ perfusion of rat livers suggested that BOPTA uptake is mediated by rodent OATP transporters. In vitro experiments revealed that BOPTA was transported by rOATP1A1, rOATP1A4, rOATP1B2 and by rMRP2 (Planchamp et al., 2007).

3.2 Kidney transporters

Renal clearance is a major pathway of drug elimination, which is the result of three concurrent processes occurring in the nephron, including glomerular filtration, tubular secretion and tubular reabsorption. Glomerular filtration is a passive process, while tubular secretion and reabsorption involve drug transporters located in the basolateral and luminal membranes of the tubular epithelium (Yin & Wang, 2016). In humans, the major transporters involved in tubular secretion of drugs include OCT2 (*SLC22A2*), OAT1 (*SLC22A6*), OAT3 (*SLC22A8*) and OATP4C1 (*SLCO4C1*) in the basolateral membrane and the multidrug and toxin extrusion (MATE) proteins MATE1 (*SLC47A1*) and MATE2-K (*SLC47A2*), P-gp, MRP2 and MRP4 (*ABCC4*) in the apical membrane (Giacomini et al., 2010). Several other related transporters are present in the proximal tubules and may also play a role in the renal elimination of drugs and drug metabolites (Yin & Wang, 2016).

Radionuclide imaging of the kidneys aims at detecting the pathophysiology of the diseased kidneys and at achieving an early and sensitive diagnosis (Szabo et al., 2011). Hippurate is a toxic urate uremic toxin that accumulates during chronic renal failure. In the past, the renal secretion of the exogenous aryl amine derivatives para-aminohippurate and the radiolabeled O-^[131I]iodohippurate were used as markers of tubular function in patients (Lowenstein & Grantham, 2016). OAT1 was shown to be the main transporter for para-aminohippurate at the proximal tubule (Vallon et al., 2008). Radiolabeled derivatives of hippurate such as p-^[18F]fluorohippurate have been proposed as PET radioligands with improved imaging performance (Awasthi et al., 2011). Today, ^[99mTc]MAG3 (mercaptoacetyl triglycine) clearance from kidneys is used in clinics to investigate tubular function in patients (Taylor, 2014). ^[99mTc]MAG3 is highly protein-bound and was shown to be cleared from plasma primarily by OAT1 in the kidneys (Shikano et al., 2004).

Furosemide, probenecid and ibuprofen were shown to be competitive inhibitors of renal OAT1 which impact [^{99m}Tc]MAG3 renography (Szabo et al., 2011). Renal secretion of the P-gp substrate [^{99m}Tc]sestamibi was shown to be inhibited by PSC833, a cyclosporine A derivative developed as a P-gp inhibitor (Luker et al., 1997).

Several PET radiotracers have been developed to investigate other specific transport systems in the kidneys. In the kidney, metformin is transported into the proximal tubule via OCT2 and excreted into the urine via MATE1 and MATE2-K (Hume et al., 2013). Pedersen et al. performed [^{11}C]metformin PET imaging in a mouse model of chronic kidney disease and suggested a contribution of mOCT2 in controlling the influx rate constant of [^{11}C]metformin into the renal cortex (Pedersen et al., 2016). In pigs, renal clearance of [^{11}C]metformin was approximately 3 times the glomerular filtration rate, demonstrating tubular secretion (Jakobsen et al., 2016). Takano and co-workers showed that sulpiride is a substrate of OCT1 (*SLC22A1*), OCT2, MATE1, and MATE2-K and proposed [^{11}C]sulpiride as a PET probe to study renal OCT function in humans (Takano et al., 2017).

3.3 Brain transporters

ABC and SLC transporters are expressed in the luminal and abluminal membranes of brain capillary endothelial cells forming the BBB, where they control access of exogenous and endogenous molecules from blood into brain parenchyma (Abbott et al., 2010; Stieger & Gao, 2015). Among different ABC and SLC transporters expressed at the BBB, P-gp is the most widely studied transporter. Previous review articles have given detailed overviews of radiotracers for imaging of P-gp at the BBB (Luurtsema et al., 2016; Mairinger et al., 2011; Raaphorst et al., 2015; Wanek et al., 2013). The applicability of the SPECT radioligand [^{99m}Tc]sestamibi for imaging P-gp function at the BBB is limited due to its very low BBB permeability, even in the absence of P-gp function (Cattelotte et al., 2009). Racemic [^{11}C]verapamil, (*R*)-[^{11}C]verapamil (Fig. 2) and [^{11}C]N-desmethyl-loperamide are PET tracers which are transported by P-gp and not by BCRP (Kannan et al., 2010; Luurtsema et al., 2003; Römermann et al., 2013) and which have been used to

visualize P-gp function at the rodent and human BBB in various settings. These radiotracers were very useful in measuring the effects of P-gp inhibition at the BBB with inhibitors such as cyclosporine A and tariquidar (Bauer et al., 2012; Damont et al., 2016; Kreisl et al., 2015; Sasongko et al., 2005). Figure 4 shows PET data on the effect of the third-generation P-gp inhibitor tariquidar on brain distribution of (*R*)-[¹¹C]verapamil in rats and in humans.

Beside the imaging of P-gp, efforts have also been directed towards the development of PET tracers for other ABC transporters at the BBB. Quantitative targeted absolute proteomics work indicated that BCRP is the most abundant ABC transporter at the human BBB (Uchida et al., 2011). The development of BCRP-selective PET tracers is hindered by the overlapping substrate specificities between BCRP and P-gp. The few BCRP-selective compounds tested as PET tracers to date all possessed similar brain kinetics in wild-type and BCRP knockout mice or rats, suggesting lack of suitability to measure BCRP function at the BBB (Hosten et al., 2013; Mairinger et al., 2010; Sivapackiam et al., 2016; Takashima et al., 2013). Wanek and co-workers developed a PET protocol for visualization of BCRP function at the BBB based on PET scans with the dual P-gp/BCRP substrate [¹¹C]tariquidar, in which unlabeled tariquidar is co-administered to inhibit P-gp at the BBB (Wanek et al., 2012). Okamura et al. developed 6-bromo-7-[¹¹C]methylpurine as a PET tracer to measure the function of multidrug resistance-associated protein (MRP1, *ABCC1*) in the brain. The PET tracer crosses the BBB, presumably by passive diffusion, and is converted inside the brain by glutathione-S-transferases into its glutathione conjugate, which leaves the brain by MRP1 efflux (Okamura et al., 2009). While this radiotracer showed great differences in brain clearance between wild-type and *Abcc1*^(-/-) mice, the exact site in the brain at which MRP1 contributes to radioactivity elimination (e.g. choroid plexus, glia cells or brain capillary endothelial cells) has not yet been fully established. Galante and colleagues recently synthesized a ¹⁸F-labeled version of this MRP1 tracer (Galante et al., 2014) for which brain clearance was also shown to be dependent on mMRP1 function.

In addition to efflux transporters, uptake transporters are also expressed at the BBB. Several studies have provided evidence of a novel and molecularly unknown drug/proton (H^+)-antiporter that controls the permeation of many CNS-targeted drugs across the BBB (e.g. cocaine, clonidine, nicotine, oxycodone and diphenhydramine). Two studies have attempted to measure the function of this novel transporter by PET imaging. Auvity et al. used the radiolabeled histamine H_1 -receptor antagonist [^{11}C]diphenhydramine to image this transporter at the rat BBB (Auvity et al., 2017). An evaluation of radiotracers for the H^+ -antiporter is complicated by a lack of suitable inhibitors for in vivo use. Auvity et al. therefore demonstrated with in situ brain perfusion that uptake transport of [^{14}C]diphenhydramine into rat brain can be inhibited by a large excess of unlabeled diphenhydramine (10 mM). In addition, they demonstrated poor brain penetration of radiolabeled metabolites, allowing for accurate quantification of CL_{uptake} from blood into brain from early PET data. Gustafsson et al. performed combined PET imaging and microdialysis in rats with [^{11}C]oxycodone, which is also a substrate of the H^+ -antiporter (Gustafsson et al., 2017). However, the utility of this radiotracer for transporter imaging is most likely limited due to extensive metabolism and brain uptake of radiolabeled metabolites. To our knowledge, no attempts have so far been made to image OATP1A2 and OATP2B1 at the BBB, the latter being considered as target transporters for facilitating brain entry of therapeutic drugs (Stieger & Gao, 2015). A potential complication in the development of PET tracers for these uptake transporters may be the fact that known transporter substrates are also recognized by efflux transporters at the BBB, which may mask the effect of the uptake transporters (Taskar et al., 2017; Tournier et al., 2013).

3.4 Retina transporters

Drug distribution to the retina after systemic administration is controlled by the blood-ocular barriers, i.e. the blood-aqueous humor barrier and the blood-retinal barrier (BRB) (Hosoya et al., 2011). The BRB is composed of retinal capillary endothelial cells (inner BRB) and retinal pigmented epithelial cells (outer BRB) (Mannermaa et al., 2006). Similar to brain capillary endothelial cells, these cells are linked by tight junctions, which may limit paracellular diffusion of drugs. The cells

comprising the BRB express SLC and ABC transporters, which may control the delivery of drugs from blood to the retina (Hosoya et al., 2011; Mannermaa et al., 2006; Stieger & Gao, 2015). P-gp was shown to be localized at both the luminal membrane of retinal capillary endothelial cells and at the apical and basolateral membranes of retinal pigmental epithelial cells (Hosoya et al., 2011; Mannermaa et al., 2006).

Invasive experiments in rodents have revealed the functional impact of P-gp at this specific barrier (Chapy et al., 2016). Compared with the BBB, mBCRP-mediated efflux was shown to be less involved at the BRB, whereas mMRPs were involved to a similar degree at both barriers (Chapy et al., 2016). Using (*R*)-[¹¹C]verapamil PET imaging in healthy volunteers, Bauer et al. reported a significant increase in radiotracer distribution to the retina during P-gp inhibition, which provided first in vivo evidence for P-gp transport activity at the human BRB. Interestingly, the increase in retinal distribution was quantitatively less pronounced than for the brain (Bauer et al., 2017a), which correlated well with preclinical data obtained in rodents (Chapy et al., 2016).

3.5 Lung transporters

Oral inhalation of therapeutic aerosols is used as a modality for local treatment of respiratory diseases (i.e. asthma and chronic obstructive pulmonary disease), but also as a route for systemic delivery of small molecule drugs and biologicals. The pharmacokinetic behavior of inhaled drugs is much more complicated than that of traditional routes of administration, with several sources of variability. There is accumulating evidence that membrane transporters belonging to the SLC and ABC families (e.g. organic cation / carnitine transporter 1 and 2 (OCTN1/2, *SLC22A4/5*), OCT1-3, MRP1, P-gp and BCRP) are expressed in bronchial, bronchiolar and alveolar epithelial cells forming the air-blood barrier (Nickel et al., 2016; Sakamoto et al., 2013). Drug transporters may be a source of variability in pulmonary PK of inhaled drugs and thus contribute to heterogeneity in treatment response and occurrence of systemic side effects (Nickel et al., 2016). PET and SPECT can be used to assess pulmonary disposition of inhaled radiopharmaceuticals (Dolovich & Labiris, 2004) and may thus also be of use to study the function of pulmonary transporters when

radiolabeled transporter substrates are employed. So far, only very few studies have used SPECT or PET imaging to investigate pulmonary transporters. Okamura and colleagues reported that pulmonary elimination of radioactivity following intravenous injection of 6-bromo-7- $[^{11}\text{C}]$ methylpurine is markedly reduced in *Abcc1*^(-/-) mice as compared with wild-type mice, suggesting that this radiotracer can be used to measure pulmonary mMRP1 function (Okamura et al., 2013). Two other studies used SPECT imaging to assess pulmonary disposition of inhaled $[^{99\text{m}}\text{Tc}]$ sestamibi, a substrate of P-gp and MRP1, in humans (Perek et al., 2000; Piwnicka-Worms et al., 1993). Ruparelia et al. reported delayed pulmonary elimination of inhaled $[^{99\text{m}}\text{Tc}]$ sestamibi in smokers versus non-smokers and hypothesized that this was due to a smoke-induced up-regulation of pulmonary P-gp (Ruparelia et al., 2008). Mohan et al. confirmed delayed $[^{99\text{m}}\text{Tc}]$ sestamibi elimination from the lungs of smokers versus non-smokers and more importantly found a correlation between $[^{99\text{m}}\text{Tc}]$ sestamibi elimination and a semi-quantitative measure of MRP1 expression in surgically resected lung tissue (Mohan et al., 2016).

3.6 Placenta transporters

High expression levels of several ABC and SLC transporters have been reported in the placenta. These transporters may play a role in controlling the distribution of drugs across the placental barrier (Nishimura & Naito, 2005, 2008). The use of nuclear imaging during pregnancy is restricted due to the lack of knowledge regarding the impact of ionizing radiation exposure on the developing fetus. However, PET imaging has been proposed to investigate drug exposure to the fetus in gestating non-human primates (Benveniste et al., 2003). Eyal and coworkers used $[^{11}\text{C}]$ verapamil PET imaging in gestating macaques to study the role of P-gp at the placental barrier (Eyal et al., 2009a). Inhibition of P-gp using cyclosporine A dose-dependently increased $[^{11}\text{C}]$ verapamil uptake by the fetal brain and liver (Ke et al., 2013). These studies convincingly demonstrated the role of P-gp in limiting the placental permeation of its substrates in vivo.

3.7 Imaging transporter function in cancer tissues

Imaging techniques have provided convincing evidence for transporter-mediated multidrug resistance (MDR) in cancer tissues. The widespread availability of [^{99m}Tc]sestamibi as a SPECT tracer in clinical routine allowed for its straightforward application in cancer patients to measure P-gp overexpression in tumors in vivo and predict MDR (Derebek et al., 1996). In patients, P-gp inhibition resulted in a significant increase in the uptake (Agrawal et al., 2003; Chen et al., 1997) and, more importantly, a decrease in the wash-out rate of [^{99m}Tc]sestamibi in tumors (Taki et al., 1998), thus highlighting the functional impact of this transporter on the distribution of its substrates to cancer tissues. [^{99m}Tc]tetrofosmin, another cationic cardiac imaging agent, has been evaluated as an alternative P-gp substrate to predict MDR in cancer (Kao et al., 2001). [^{99m}Tc]sestamibi and [^{99m}Tc]tetrofosmin are not transported by BCRP (Tournier et al., 2009) but are both substrates of MRP1, which limits their utility as P-gp specific imaging agents in cancer (Perek et al., 2000). P-gp substrates radiolabeled with positron emitting isotopes have been further proposed to study MDR using PET (Mann et al., 2015). [^{11}C]Verapamil PET imaging has been performed in cancer patients, but failed to provide clinical utility (Eary et al., 2011; Hendrikse & Vaalburg, 2002). Radiolabeled analogs or derivatives of taxanes such as [^{11}C]docetaxel and [^{18}F]fluoropaclitaxel have been evaluated in cancer patients as predictors of successful therapy (Kurdziel et al., 2011; van der Veldt et al., 2013a, 2013b).

Dysregulation of SLC transporters appears to be a common feature in many tumors (El-Gebali et al., 2013). In hepatocellular carcinoma, the expression of OATP1B3 decreases in parallel with tumor differentiation (Kitao et al., 2011; Miura et al., 2015; Yoneda et al., 2013). This decreased expression was found to be paralleled by a reduced gadoxetate-induced signal enhancement in the tumor (Tsuboyama et al., 2010). A correlation has been found between the expression of OATP1B1 and/or OATP1B3 in pathologic liver cells and signal enhancement (Tsuboyama et al., 2010). Focal nodular hyperplasia and hepatocellular adenoma are usually difficult to distinguish in the absence of contrast enhancement. This distinction is, however, highly important clinically

(Pastor et al., 2014). Again, a correlation between OATP1B3 expression and intensity of the enhancement was found (Doi et al., 2011; Yoneda et al., 2016). The same tumors may, however, also express an efflux system for the contrast agent, e.g. MRP2 or MRP3 (Kitao et al., 2010; Thian et al., 2013; Tsuboyama et al., 2010; Vilgrain et al., 2016). These examples highlight the limitations of determining in vivo transporter expression/function using imaging agents which are not specific to an individual transporter. An alternative approach to distinguish between these two lesions may be PET imaging with [^{18}F]fluorocholine. Three patients with hepatocellular adenoma were found to show reduced [^{18}F]fluorocholine uptake into the lesion, which correlated with a reduced expression of OCT1 and OCT3 (Visentin et al., 2017).

4 Discoveries and perspectives enabled by transporter imaging

4.1 Implications of the interplay between transporters and metabolism for imaging

As the liver is a central organ in drug metabolism and elimination, defining the transport systems involved in these processes as well as understanding the role of these transporters in the PK of drugs is a very active field of research (Burt et al., 2015; Hillyar et al., 2015; Testa et al., 2015). The interplay between transport and metabolism is termed metabolic channeling (Srere & Ovadi, 1990). The channeling of drugs via transporters into metabolism has been known of at the phenomenological level in clinics for quite some time and demonstrated by means of pharmacokinetic DDI studies (Benet, 2009; Custodio et al., 2008). One study tested the interaction of rifampicin with glyburide disposition in two settings: a single dose of rifampicin led to an increase of systemic glyburide exposure, while multiple dosing of rifampicin led to a considerable reduction of glyburide exposure (Zheng et al., 2009). Tournier et al. mimicked this glyburide-rifampicin interaction using [^{11}C]glyburide PET imaging in non-human primates. Single dose rifampicin resulted in a 14-fold decrease in [^{11}C]glyburide uptake by the liver. Reduced liver uptake was associated with a pronounced 5-fold increase in [^{11}C]glyburide exposure in plasma. Interestingly, radiolabeled metabolites, which accounted for ~30% of total radioactivity in plasma, could not be detected in plasma after OATP inhibition (Tournier et al., 2013) (Fig. 5). Imaging may thus be

useful to investigate the interplay between transporter-mediated liver uptake and metabolic enzymes in controlling the liver metabolism of drugs. Metabolic channeling is gaining attention in the drug development and pharmacokinetic community (Varma et al., 2015; Varma & El-Kattan, 2016). An additional level of complexity is added by the fact that drug metabolites may also interact with (efflux) transporters, as exemplified for bosentan (Fattinger et al., 2001) and troglitazone (Funk et al., 2001). The issues of metabolic flux and transporter-metabolism interplay have, to our knowledge, not been specifically addressed at the mechanistic level with imaging probes in different organs.

4.2 Imaging disease-induced changes in transporter expression at the BBB

Several PET studies have been conducted using racemic [^{11}C]verapamil or (*R*)-[^{11}C]verapamil to investigate cerebral P-gp function in different patient groups. However, due to the low sensitivity of [^{11}C]verapamil to measure moderate changes in P-gp expression/function at the BBB (Wanek et al., 2015), most studies found only small differences between patients and control groups. A pilot PET study with (*R*)-[^{11}C]verapamil in seven patients with unilateral temporal lobe epilepsy failed to reveal significant differences in (*R*)-[^{11}C]verapamil distribution between epileptic brain tissue and contralateral healthy brain tissue (Langer et al., 2007). Van Assema et al. found no differences in (*R*)-[^{11}C]verapamil volume of distribution (V_T) and K_1 values, which were shown in previous studies to be the key parameters associated with P-gp function at the BBB (Muzi et al., 2009; Wagner et al., 2009) between Alzheimer's disease (AD) patients and age-matched control subjects (van Assema et al., 2012a). On the other hand, the authors found a significantly higher non-displaceable binding potential (BP_{ND}) of (*R*)-[^{11}C]verapamil in AD patients, which they hypothesized to reflect a decrease in cerebral P-gp function (van Assema et al., 2012a). In a study by Deo et al., differences in brain distribution of [^{11}C]verapamil between AD patients and control subjects could only be detected when regional [^{11}C]verapamil K_1 values were normalized to regional cerebral blood flow, which was lower in AD patients than in control subjects (Deo et al., 2014). These data suggest that brain distribution of [^{11}C]verapamil depends on cerebral blood

flow, as shown before for [^{11}C]N-desmethyl-loperamide (Kreisl et al., 2010; Liow et al., 2009). A series of studies used statistical parametrical mapping (SPM) analysis to investigate regional differences in brain distribution of racemic [^{11}C]verapamil in Parkinson's disease and depression and schizophrenia patients (Bartels et al., 2008; de Klerk et al., 2009, 2010; Kortekaas et al., 2005). These studies found small regional differences in [^{11}C]verapamil brain distribution, which were attributed to regional differences in P-gp function. There is evidence that P-gp expression at the BBB not only changes in the course of different neurological diseases, but also during healthy ageing. This could be of therapeutic relevance, as elderly people often take many different drugs which could show altered brain distribution if their brain distribution were limited by P-gp. A few studies used racemic [^{11}C]verapamil or (*R*)-[^{11}C]verapamil to compare cerebral P-gp function between elderly and young volunteers (Bartels et al., 2009; Bauer et al., 2009; Toornvliet et al., 2006; van Assema et al., 2012b). These studies consistently found small increases in radiotracer brain distribution, both on a global and regional level, in elderly versus young subjects, which pointed to an age-related, moderate decline in P-gp function at the BBB. A few studies employed a partial P-gp inhibition protocol, which showed improved sensitivity to measure moderate changes in P-gp expression/function at the BBB than baseline PET scans (Bankstahl et al., 2011). Feldmann et al. demonstrated that patients with pharmacoresistant unilateral temporal lobe epilepsy had a lower increase in (*R*)-[^{11}C]verapamil uptake in the ipsilateral hippocampus in response to tariquidar administration than healthy control subjects (24.5% vs. 65% increase in (*R*)-[^{11}C]verapamil K_1) (Feldmann et al., 2013). This attenuated response to tariquidar was consistent with increased P-gp expression in the hippocampus of pharmacoresistant epilepsy patients. Another study used cyclosporine A for partial P-gp inhibition at the BBB and found a significantly higher asymmetry in (*R*)-[^{11}C]verapamil brain concentrations between ipsilateral and contralateral temporal lobe brain regions of drug-resistant temporal lobe epilepsy patients as compared with drug-sensitive epilepsy patients and healthy control subjects (Shin et al., 2016). Bauer et al. demonstrated that the partial P-gp inhibition protocol can also be used to visualize a

reduction of P-gp at the BBB as occurs during healthy ageing (Bauer et al., 2017c). They found a significantly higher increase in (*R*)-[¹¹C]verapamil whole-brain V_T in elderly versus young subjects (+40% vs. + 2%) in response to tariquidar administration. However, the partial P-gp inhibition protocol is difficult to use in patients due to safety concerns associated with P-gp inhibition and the concomitant use of medication. Clearly, to measure P-gp function at the BBB there is still a need for more sensitive PET tracers which do not require administration of a P-gp inhibitor and which lack brain-penetrant radiolabeled metabolites.

4.3 Imaging drug-drug interactions at the tissue level

Beside drug metabolizing enzymes, transporters may also be implicated in DDIs. In transporter-mediated DDIs, concomitant administration of two drugs which interact with the same transporters may lead to changes in the PK of the drugs as compared to when these drugs are dosed alone, due to mutual inhibition or saturation of ABC and SLC transporters controlling drug tissue distribution or excretion (Giacomini et al., 2010; Lee et al., 2017). Such DDIs can seriously affect drug safety and efficacy (König et al., 2013), and regulatory authorities therefore require assessment of the DDI risk of new drug candidates (EMA, 2013; FDA, 2017). In many cases, transporter-mediated DDIs lead to changes in drug plasma PK and can therefore be detected by monitoring plasma concentrations of drugs. In some cases, however, inhibition of transporters may predominantly affect drug tissue concentrations (Kusuhara & Sugiyama, 2009; Patilea-Vrana & Unadkat, 2016). For instance, inhibition of efflux transporters in the canalicular membrane of hepatocytes or in the brush border membrane of kidney proximal tubule cells may lead to changes in liver and kidney concentrations of drugs. Nuclear imaging methods allow assessment of tissue concentrations of radiolabeled drugs and have therefore been proposed as a tool to investigate such silent DDIs (Kusuhara, 2013; Langer, 2016; Wulkersdorfer et al., 2014). Consequently, several groups have developed radiolabeled probe substrates for measuring the activities of ABC and SLC transporters in different organs with PET (e.g. brain, liver and kidney) (Langer, 2016;

Marie et al., 2017; Testa et al., 2015). Most of these probes lack selectivity for a single ABC or SLC transporter.

Japanese researchers around Prof. Yuichi Sugiyama developed several PET probe substrates to measure the activities of sinusoidal uptake transporters in hepatocytes ((15*R*)-[¹¹C]TIC-Me, [¹¹C]telmisartan, [¹¹C]dehydropravastatin) (Kusuhara, 2013). (15*R*)-[¹¹C]TIC-Me is a methyl ester which is rapidly converted into its free acid (15*R*)-[¹¹C]TIC in blood. In one study it was shown that $CL_{\text{uptake, liver}}$ and $CL_{\text{int, bile}}$ of (15*R*)-[¹¹C]TIC-associated radioactivity were reduced in healthy human subjects after oral treatment with rifampicin, which was attributed to inhibition of sinusoidal OATPs (OATP1B1, OATP1B3) and possibly canalicular MRP2 (Takashima et al., 2012). Similar results were obtained with [¹¹C]dehydropravastatin after rifampicin treatment in humans (Kaneko et al., 2018). Metformin is a widely used oral antidiabetic drug. PET studies with [¹¹C]metformin revealed pronounced changes in liver and kidney distribution of [¹¹C]metformin in rodents when uptake transporters (mOCT1 and mOCT2) and efflux transporters (mMATE) were pharmacologically inhibited with cimetidine and pyrimethamine, respectively (Shingaki et al., 2015; Jensen et al., 2016). The radiolabeled epidermal growth factor receptor-targeting tyrosine kinase inhibitor (TKI) [¹¹C]erlotinib was found to be transported in vitro at low concentrations as used for PET imaging by OATP2B1, but not by OATP1B1 and OATP1B3, whereas OATP2B1 transport was saturated at pharmacologic erlotinib concentrations. In vivo, liver uptake of [¹¹C]erlotinib was markedly lower after pre-treatment of healthy volunteers with a therapeutic erlotinib dose as compared with administration of a microdose of [¹¹C]erlotinib, which appeared to be caused by saturation of OATP2B1 transport (Bauer et al., 2017b) (Fig. 6). These data suggested that erlotinib may be a perpetrator of OATP2B1-mediated DDIs when combined with drugs which are mainly taken up into the liver by OATP2B1. Moreover, [¹¹C]erlotinib may be useful as an OATP2B1-specific probe substrate for PET studies.

The SPECT tracer [^{99m}Tc]mebrofenin is a substrate of OATP1B1, OATP1B3, MRP2 and MRP3 (de Graaf et al., 2011; Ghibellini et al., 2008). Pfeifer et al. demonstrated in a SPECT study in

healthy volunteers that oral pretreatment with ritonavir decreased $CL_{\text{uptake,liver}}$ of [^{99m}Tc]mebrofenin by 46%, which was attributed to inhibition of OATP1B1 and OATP1B3 (Pfeifer et al., 2013).

The entry of gadoxetate and BOPTA into hepatocytes is mediated by OATP1B1 and OATP1B3, and they are secreted into bile in unchanged form through MRP2. Kato et al. investigated the interaction between gadoxetate and 11 clinically used drugs in rats using MRI (Kato et al., 2002). They found a significant decrease in contrast enhancement of the liver when rats were pre-treated with rifampicin, but concluded that a combination of gadoxetate and rifampicin is unlikely to occur in the clinic. One study in healthy volunteers investigated the interaction between gadoxetate and the macrolide antibiotic erythromycin, which inhibits OATP1B1 and OATP1B3 in vitro (Seithel et al., 2007), and failed to detect a significant effect of erythromycin on liver enhancement (Huppertz et al., 2011).

PET imaging has been useful in evaluating the risk for DDIs at the level of ABC transporters expressed at the BBB, which may lead to increased brain distribution of drugs and CNS side effects (Eyal et al., 2009b). In a seminal position paper, Kalvass and colleagues argued that transporter-mediated DDIs at the BBB are unlikely to occur in clinical practice as most marketed drugs do not achieve high enough unbound plasma concentrations to lead to significant efflux transporter inhibition at the BBB (Kalvass et al., 2013). Exceptions are the immunosuppressant drug cyclosporine A, which was shown to increase brain exposure of [^{11}C]verapamil 1.8-fold when administered as an intravenous infusion concurrent with the PET scan (Sasongko et al., 2005). The antiarrhythmic agent quinidine was also shown to exert a small P-gp inhibitory effect at the human BBB at clinically relevant plasma concentrations (Liu et al., 2015). In contrast to the majority of marketed drugs, several non-marketed, experimental P-gp inhibitors which were originally developed as MDR reversal agents (e.g. elacridar, tariquidar and valspodar) (Szakács et al., 2006) were shown to lead to significant increases in brain distribution of P-gp substrates. For instance, up to 4- to 5-fold increases in brain distribution of (*R*)-[^{11}C]verapamil or [^{11}C]N-desmethyl-loperamide were observed in healthy volunteers when tariquidar was intravenously

infused during the PET scan (Bauer et al., 2015; Kreisl et al., 2015). These studies do not represent realistic scenarios for clinically relevant transporter-mediated DDIs at the BBB as they employed non-marketed drugs dosed intravenously at rather high doses. Nevertheless, these data suggest that efflux transporter inhibition at the human BBB is in principle feasible and may be exploited to enhance brain distribution of drugs targeted to the brain.

4.4 Imaging the impact of transporter gene polymorphisms on transporter function

Polymorphisms in the genes encoding transport proteins were shown to be associated with alterations in transporter activity and expression, leading to inter-individual variability in drug disposition and drug response (Ieiri, 2012; König et al., 2006; Maeda & Sugiyama, 2008; Niemi et al., 2011). Imaging with radiolabeled transporter substrates is an obvious way to assess the functional impact of these genetic variants in different tissues, and a handful of clinical studies have so far been published.

Two studies assessed the impact of a combination of three different *ABCB1* SNPs on brain distribution of racemic [¹¹C]verapamil (Brunner et al., 2005; Takano et al., 2006). Both studies failed to detect differences in [¹¹C]verapamil brain distribution between healthy homozygous carriers of the TTT haplotype (c.3435T, c.2677T, and c.1236T) and homozygous carriers (controls) of the CGC haplotype (c.3435C, c.2677G, and c.1236C). Interestingly, a study in AD patients by van Assema found an association between T dose in the c.C1236T, c.G2677T and c.C3435T SNPs and BP_{ND} of (*R*)-[¹¹C]verapamil (van Assema et al., 2012c). Carriers of the variant alleles had a higher BP_{ND} than non-carriers, which was interpreted by the authors as decreased P-gp function at the BBB. The same study found no effect of these *ABCB1* SNPs on brain distribution of (*R*)-[¹¹C]verapamil in healthy control subjects, which confirmed the results obtained by Brunner and Takano (Brunner et al., 2005; Takano et al., 2006) and suggested that genetic variations in the *ABCB1* gene might affect P-gp function or expression at the BBB only when P-gp function is already compromised. The most important SNP in the *ABCG2* gene is c.421C>A. This non-synonymous SNP has been associated with diminished expression of BCRP in various

tissues, such as the liver, placenta and intestine (Kobayashi et al., 2005; Prasad et al., 2013) and with changes in plasma PK of BCRP substrate drugs, such as sulfasalazine and rosuvastatin (Keskitalo et al., 2009; Yamasaki et al., 2008). Bauer et al. used a newly developed PET protocol (Wanek et al., 2012) to measure the impact of this *ABCG2* SNP on the function of BCRP at the BBB (Bauer et al., 2016). This PET protocol is based on PET scans with the dual P-gp/BCRP substrate [^{11}C]tariquidar, in which unlabeled tariquidar is co-administered to inhibit P-gp and eliminate the contribution of P-gp to brain distribution of the dual substrate. Bauer reported 1.7-fold higher [^{11}C]tariquidar V_T values in heterozygous SNP carriers (c.421CA) as compared with non-carriers (c.421CC), which was consistent with reduced BCRP transport activity at the BBB of SNP carriers (Bauer et al., 2016). Interestingly, no difference in [^{11}C]tariquidar brain distribution was observed between carriers and non-carriers in PET scans without tariquidar co-administration, which indicated that the effect of the *ABCG2* SNP was masked in presence of functional P-gp. Variants in the *SLC22A1* gene, which encodes OCT1, were shown to affect OCT1 function and expression and have been associated with altered response to the oral antidiabetic drug metformin compared with carriers of the reference allele, without affecting metformin systemic exposure (Zamek-Gliszczynski et al., 2017). This is because the liver is the target organ of metformin distribution and pharmacologic activity, but the drug is ultimately eliminated by the kidney. Sundelin et al. showed that exposure of [^{11}C]metformin in the liver was significantly lower in carriers of p.M420del and p.R61C variants in *SLC22A1* after both oral and intravenous administration of the PET tracer (Sundelin et al., 2017). This supported the notion that genetic *SLC22A1* variants may affect metformin response. Two studies found diminished liver enhancement with gadoxetate in carriers of *SLCO1B1* SNPs without changes in plasma PK of gadoxetate, suggesting that genetic transporter variants may be signal confounders in gadoxetate-enhanced diagnostic liver MRI (Nassif et al., 2012; Okubo et al., 2013). One study examined liver distribution of [$^{99\text{m}}\text{Tc}$]mebrofenin in healthy volunteers and patients with nonalcoholic steatohepatitis and found a diminished $CL_{\text{uptake,liver}}$ of [$^{99\text{m}}\text{Tc}$]mebrofenin in healthy

volunteers who were carriers of low/intermediate function *SLCO1B1* genetic variants (Ali et al., 2017). It can be expected that transporter polymorphisms will also affect tissue uptake of other clinically used diagnostic radiotracers or contrast agents and should therefore be considered in the interpretation of imaging data.

4.5 Animal to clinical extrapolation

Species differences have been reported in the substrate recognition and transport efficacy of ABC and SLC transporters (Kim et al., 2008; Li et al., 2008; Xia et al., 2006; Yamazaki et al., 2001). For instance, it has been shown that antiseizure drugs are good substrates of mouse P-gp (mABCB1A), whereas they are only weakly transported by human P-gp (Baltes et al., 2007; Luna-Tortós et al., 2008). The PET tracer [¹⁸F]MPPF (4-(2'-methoxyphenyl)-1-[2'-(N-2"-pyridinyl)-p-[¹⁸F]fluorobenzamido]ethylpiperazine) was shown to be transported by mABCB1A, but not by human P-gp (Tournier et al., 2011, 2012). Direct murine orthologues do not exist for some drug transporters. For example, in the sinusoidal membrane of human hepatocytes the *SLCO* transporters OATP1B1, OATP1B3 and OATP2B1 are expressed, whereas in mouse hepatocytes mOATP1B2, mOATP2B1, mOATP1A1 and mOATP1A4 are expressed (Durmus et al., 2016). mOATP1B2 is the mouse orthologue of human OATP1B1 and OATP1B3. mOATP1A1 and mOATP1A4 are mouse orthologues of human OATP1A2, which in the human liver is not expressed in the sinusoidal hepatocyte membrane but in epithelial cells of the bile duct. Owing to these differences, preclinical data on OATP-mediated transport in rodent liver is notoriously difficult to extrapolate to humans (Durmus et al., 2016) (Fig. 6). The advent of quantitative targeted absolute proteomics has provided a tool to quantify absolute expression levels of transport proteins in different organs and tissues (Ohtsuki et al., 2011). These studies, too, revealed pronounced species differences in transporter expression. For instance, while BCRP was shown to be the most abundant ABC transporter at the human BBB, P-gp (mABCB1A) was found to be more abundant at the mouse BBB than mBCRP (Shawahna et al., 2011; Uchida et al., 2011). While mMRP4 was detected as the third most abundant ABC transporter at the mouse BBB, it has

not yet been detected at the human BBB (Uchida et al., 2011). Such species differences are expected to lead to species differences in tissue distribution and excretion of PET/SPECT radiotracers and MRI contrast agents which are transporter substrates. Owing to the availability of dedicated small-animal PET, SPECT and MRI systems, differences between rodents and humans can be directly investigated in a translational approach using the same technology. However, several caveats have to be kept in mind. One important issue is the fact that imaging studies in rodents are usually performed under anaesthesia (e.g. isoflurane), while this is not the case in humans. **Second, it is well known that species differences also exist in metabolism pathways of xenobiotics (Martignoni et al., 2006). Therefore, species differences in radiotracer metabolism may mask species differences in drug transporters.** Third, several key physiological parameters differ between rodents and humans (e.g. organ blood flow) (Davies & Morris, 1993), which may also exert an influence on tissue distribution of radiotracers. Some imaging studies have attempted to address these issues specifically. Syvänen et al. assessed differences between rats, monkeys and humans in brain distribution of three radiolabeled P-gp substrates ($[^{11}\text{C}]$ verapamil, $[^{11}\text{C}]$ GR205171 and $[^{18}\text{F}]$ altanserin). These authors found higher brain-to-plasma ratios of radioactivity in monkeys or humans as compared with rats, and higher increases in brain distribution of these radiotracers following cyclosporine A treatment in rats than in monkeys (Syvänen et al., 2009). Similarly, P-gp inhibition with tariquidar at comparable tariquidar plasma concentrations produced a higher increase in brain uptake of (*R*)- $[^{11}\text{C}]$ verapamil in rats than in humans (Kuntner et al., 2010; Bauer et al., 2012; Wagner et al., 2009) (Fig. 4). In accordance with this, in vivo half-maximum inhibitory concentrations for enhancement of brain uptake of (*R*)- $[^{11}\text{C}]$ verapamil by tariquidar were determined to be lower in rats (873 nM) than in humans (2,248 nM) (Bauer et al., 2015; Kuntner et al., 2010) (Fig. 4). This stands in contrast with data for cyclosporine A, for which increases in brain uptake of $[^3\text{H}]$ verapamil achieved in rats were found to predict increases in brain uptake of $[^{11}\text{C}]$ verapamil in humans (Hsiao et al., 2006). Wanek et al. systematically assessed possible reasons for species differences in brain distribution of (*R*)-

[¹¹C]verapamil and [¹¹C]*N*-desmethyl-loperamide (Wanek et al., 2015). They concluded that species differences in metabolism along with brain uptake of radiolabeled metabolites precluded an assessment of species differences in P-gp transport activity at the BBB with these two radiotracers. Consequently, for an assessment of species differences in ABC and SLC transporter activities, the availability of radiotracers that are not metabolized over the duration of a PET scan in all investigated species is mandatory.

No general recommendations can be made as to which preclinical species better predicts the pharmacokinetic disposition of an imaging probe in humans. For instance, with respect to P-gp/BCRP activity at the BBB, non-human primates have been identified as a more appropriate model of the human BBB than rodents (Ito et al., 2011; Uchida et al., 2011). In recent years, efforts have been directed toward the development of mouse models humanized for certain ABC or SLC transporters or metabolizing enzymes (Choo & Salphati, 2018). These mouse models still suffer from certain limitations. In most cases, for instance, the models are only humanized for a single transporter gene and compensatory up-/down-regulation of other transporters or enzymes may occur. Nevertheless, these animal models may ultimately allow a better translation of transporter imaging results from mice to humans. Finally, it should be emphasized that PET and SPECT use sub-pharmacological doses (microdoses) of imaging probes, so that toxicity issues are not usually of concern in imaging experiments in humans. In many cases, transporter imaging probes are derived from known drugs or drug metabolites, and regulatory hurdles preventing rapid translation of PET tracers from preclinical species into humans are much smaller than in conventional drug development (ICH Expert Working Group, 2009). Consequently, while rodent experiments may be very useful in developing and improving methodological aspects and addressing certain questions, such as the tissue content of radiolabeled metabolites, small pilot studies in healthy human volunteers may be the best way to assess the suitability of a novel transporter imaging probe for clinical use.

4.6 Pharmacokinetic imaging to study the fate of drugs at the site of action

The study of drug transporters with imaging modalities most commonly relies on the use of prototypical probe substrates. These are ideally designed to be specific to one transporter with high transport capacity, in order to generate substantial “contrast” between conditions where transporter activity is absent or present (Kannan et al., 2009; Wanek et al., 2015). However, quantitative data obtained with one prototypical probe substrate for one transporter might not be readily translated to other substrates of this transporter. Indeed, xenobiotics have different affinities for transporters, and scaling factors and suitable models have to be considered to extrapolate the in vivo impact of transporters on the tissue permeation of drugs of interest (Kalvass et al., 2013; Matsuda et al., 2017). Moreover, most drugs are often substrates of multiple transporters and considerable overlap between ABC and SLC substrates is commonly observed (Gui et al., 2010; Matsson et al., 2009). Substrates of influx transporters may also undergo efflux transport at the same interface. In this situation, the overall net flux of the drug results from the competition between the influx and efflux component, which is difficult to predict in the real-life situation (Chapy et al., 2016; Taskar et al., 2017).

As an alternative to the use of prototypical probe substrates, direct isotopic labeling of the drug of interest may be employed. Theoretically, the study of drug distribution in selected organs and exposure to target/vulnerable tissues is feasible. However, careful interpretation of PET data is required to avoid any misinterpretation regarding drug exposure to investigated tissues. In most situations, radioactivity in tissues cannot be straightforwardly converted into local drug concentrations at the sites of action (Pike, 2009). Conventional pharmacokinetic studies accurately measure the free ligand in plasma and are able to detect the presence of metabolites using analytical chemistry methods. However, PET-based kinetic imaging does not distinguish between radioactivity associated with the vascular content, the free, bound, and parent compounds, and radiolabeled metabolites in tissues (Pike, 2009).

So far, pharmacologic imaging has predominantly focused on the interaction of drugs with their respective molecular targets in tissue through receptor occupancy (now named target engagement) studies (Willmann et al., 2008). Advances in PET data acquisition and modeling have considerably enhanced the applicability of imaging biodistribution studies and paved the way for what could be named pharmacokinetic imaging. When drugs are amenable to radiolabeling, it is now possible to investigate the interplay of membrane transporters in controlling the clearance and tissue distribution of drugs. Using this approach, the impact of SLC and ABC transporters has been revealed in a dynamic fashion for glyburide (Tournier et al., 2013), metformin (Jensen et al., 2016), sulpiride (Takano et al., 2017), metoclopramide (Pottier et al., 2016), ciprofloxacin (Wanek et al., 2016) and erlotinib (Bauer et al., 2017b; Traxl et al., 2015), to cite a few examples. This emerging approach is increasingly regarded as a means to elucidate the overall impact of membrane transporters on drug distribution to tissues in animals and humans (Giacomini et al., 2010). A step forward in modern pharmacology will be to investigate transporter-mediated drug uptake by tissues as a mediator of local drug exposure to tissues, and ultimately to assess or predict the contribution of membrane transporters to pharmacodynamics and toxicity. This approach is gaining attention for the study of drug delivery to sanctuary tissues, mainly in cancer. For instance, the poor CNS permeation of TKIs is now accepted as a determinant of the poor response of CNS malignancies, including brain metastasis, to this class of molecularly targeted therapy (Holohan et al., 2013; Camidge et al., 2014). PET using radiolabeled analogs of TKIs can be regarded as a useful method to compare their CNS penetration in humans (Verheijen et al., 2017) and non-human primates, a relevant animal model of the human BBB (Ballard et al., 2016; Collier et al., 2017a, 2017b). Virtually all members of the clinically important class of TKIs were identified as dual P-gp/BCRP substrates, which provides a mechanistic explanation for their low brain penetration (Agarwal et al., 2011; Durmus et al., 2015). Recent studies used [¹¹C]erlotinib PET imaging to evaluate strategies to improve the brain delivery of erlotinib. P-gp/BCRP-mediated efflux of erlotinib at the BBB was inhibited using an intravenous infusion of high-dose elacridar in

non-human primates (Tournier et al., 2017). However, P-gp/BCRP inhibition could not be achieved using an oral formulation of elacridar in humans, most likely due to the low oral bioavailability of this inhibitor (Verheijen et al., 2017).

Despite its methodological limitations, pharmacokinetic imaging is a potentially powerful approach to evaluating transporter-based strategies to improve the local delivery and/or tissue selectivity of drugs at their sites of action. Applications may not only be restricted to research in cancer therapy, but may also be extended to neurological or infectious diseases for which sanctuary sites are suspected to account for poor or incomplete therapeutic response in patients (Rizk ML et al., 2017). Moreover, radiolabeled drugs may find use in evaluating protective pharmacologic strategies to mitigate organ toxicities of certain drugs (e.g. nephrotoxicity) (Manohar & Leung, 2017; Reichart et al., 2017).

5 Expert opinion and perspectives

Imaging methods are increasingly regarded as innovative means to explore drug transporters in vivo. Advances in the development of imaging probes dedicated to the study of drug transporter function has provided new insights into the role of drug transporters at several blood-tissue interfaces. However, imaging techniques still suffer from several technical and methodological limitations, which restrict their applicability to transporter studies. Limitations related to the inability of nuclear imaging methods to distinguish parent compound from radiolabeled metabolites have been discussed in this review article. Therefore, metabolically stable probe substrates are clearly preferred for transporter imaging studies. Regulatory authorities request that pharmaceutical companies examine the interaction of new drug candidates and their metabolites with ABC and SLC transporters to assess the risk for the occurrence of DDIs (EMA, 2013; FDA, 2017). Hence, it can be assumed that a wealth of unpublished data are available on new chemical entities which never reached the market but which may prove suitable for the development of transporter imaging probes. Clearly, the field may benefit considerably from public-private partnerships between academic researchers and pharmaceutical companies in order to advance transporter imaging. A

major challenge in the imaging of transporters is related to the broadly overlapping substrate specificities of most ABC and SLC transporters, so that true transporter selectivity may be difficult to achieve in practice. However, the introduction of carefully matched, clinically feasible combinations of radiolabeled probe substrates with prototypical transporter inhibitors may prove beneficial in revealing the role played by specific ABC and SLC transporters in certain organ systems. For instance, lack of selectivity of a radiolabeled probe substrate for a given ABC or SLC transporter may be mitigated by combining it with a transporter inhibitor which inhibits only one of the ABC and SLC transporters by which the probe substrate is transported. Therefore, the screening of currently available, clinically applicable inhibitors with respect to their transporter selectivity profiles should be encouraged in order to yield effective substrate-inhibitor pairs for transporter imaging. In this framework, imaging of drug transporters may also clearly benefit from medicinal chemistry and structure-activity relationship studies aimed at improving the selectivity of transporter substrates and inhibitors (Ekins et al., 2012). It should also be noted that probe substrates which lack transporter selectivity in one organ may still prove suitable to selectively study a certain transporter in another organ. Therefore, available radiotracers should be further evaluated in organs and at blood-tissue interfaces which have not yet been the major focus of transporter studies (e.g. BRB, placenta and lungs). Another limitation in the development of transporter imaging probes is related to difficulties in extrapolating preclinical results to humans. In other words, imaging probes which were identified as suitable for transporter imaging in rodents may fail to perform in humans. This is a general problem which is also encountered in drug development. This risk may be mitigated by the introduction of more refined and better standardized preclinical assessment approaches, such as humanized mouse models, or more advanced cell culture experiments, such as sandwich-cultured hepatocytes. However, the key strength of imaging methods is that owing to their non-invasive or minimally invasive nature and the administration of very low, sub-pharmacological doses of the imaging probes, they are highly translational. Given the existence of sometimes pronounced species differences in transporters

or metabolizing enzymes, and given the current availability of several prototypical transporter inhibitors for clinical use, the best way to characterize the clinical suitability of new imaging probes may ultimately be the performance of small pilot studies in healthy human volunteers. This approach may also be very useful in the assessment of transporter-mediated DDIs of new drug candidates in early clinical drug development. However, further studies with radiolabeled probe substrates based on marketed model drugs and prototypical inhibitors are needed to refine currently available analytical methodology, such as pharmacokinetic modeling approaches, taking into account the inherent limitations of imaging approaches, and to highlight the power of this approach in drug development. Another limitation of imaging tools is their high costs and the requirement for specialized research infrastructure, in particular for PET imaging. This is where less cost-intensive imaging approaches which are better suited to a hospital environment come into play. The discovery that MRI contrast agents or SPECT tracers interact with ABC and SLC transporters was mostly serendipitous, and so far hardly any systematic efforts have been made to develop transporter-specific MRI contrast agents or SPECT tracers. Examples with re-purposed SPECT tracers such as [^{99m}Tc]mebrofenin have illustrated the power of SPECT to study transporters in vivo, particularly in view of recent advances in SPECT methodology. Further research should be therefore directed toward the development of SPECT tracers as innovative transporter imaging probes with a broader clinical applicability. To establish the clinical benefit of transporter imaging, further studies in various disease settings with currently available or newly developed imaging probes are required.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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Figure legends

Fig. 1. Relative performances of clinical imaging modalities for the study of drug transporter function in vivo. The diagram includes positron emission tomography (PET, blue), single photon emission computed tomography (SPECT, purple) and magnetic resonance imaging (MRI, orange). Scales of the individual axes are proposed for illustrative purposes only. Data and corresponding references are reported in the main text for sensitivity, quantification, temporal resolution, spatial resolution and availability. Size of the imaging probe portfolio has been estimated for each modality from the number of different agents described in the present review.

Fig. 2. Chemical structures of selected imaging agents used to study drug transporters, including the SPECT tracer [^{99m}Tc]mebrofenin (a) and the MRI contrast agent Gd-EOB-DTPA (gadoxetate) (b). Chemistry schemes for the synthesis of (*R*)-[¹¹C]verapamil by *N*-methylation of *N*-desmethyl verapamil using [¹¹C]methyl triflate (c) and 3β-[¹⁸F]fluorocholeic acid by nucleophilic ¹⁸F-fluorination of a protected mesylated precursor (d) are reported.

Fig. 3. Kinetic model of the transport of [¹¹C]cholylsarcosine (¹¹C-CSar) from blood to bile. The exchange of ¹¹C-CSar between the compartments is described by fitted parameters: K_1 (mL blood/min/mL liver tissue), k_2 (min⁻¹), k_3 (min⁻¹), and k_5 (min⁻¹). The amount of ¹¹C-CSar in the blood compartment at time t is $C_{in}(t)$ multiplied by V_{blood} (the fraction of blood in the liver tissue, mL blood/mL liver tissue). $C_{in}(t)$ is the flow-weighted mixed input of ¹¹C-CSar to the liver from the hepatic artery and the portal vein, which was calculated from the arterial blood concentrations measured by arterial blood sampling. The amount of ¹¹C-CSar in the hepatocyte compartment at time t is $C_{hep}(t)$ (concentration of ¹¹C-CSar in hepatocytes, kBq/mL hepatocytes) multiplied by V_{hep} (the fraction of hepatocytes in the liver tissue, mL hepatocytes/mL liver tissue). The amount of ¹¹C-CSar at time t in the intrahepatic bile ducts is $C_{bile}(t)$ (concentration of ¹¹C-CSar in intrahepatic bile

ducts; kBq/mL bile) multiplied by V_{bile} (the fraction of intrahepatic bile ducts in the liver tissue, mL bile ducts/mL liver tissue) (reproduced with permission from (Ørntoft et al., 2017)).

Fig. 4. PET summation images of rat (a) and human (b) brain obtained with the P-gp substrate radiotracer (*R*)-[^{11}C]verapamil at baseline and after administration of increasing doses of the third-generation P-gp inhibitor tariquidar. Radioactivity concentration is normalized to injected radioactivity amount per body weight and expressed as standardized uptake value (SUV). Concentration-response curves in rats (c) and humans (d) for enhancement of brain uptake of (*R*)-[^{11}C]verapamil (expressed as volume of distribution, V_T). Half-maximum inhibitory concentrations (IC_{50}) of tariquidar were lower in rats than in humans. Adapted from Bauer et al., 2012, 2015 and Kuntner et al., 2010.

Fig. 5. Representative [^{11}C]glyburide PET data obtained in the liver of one non-human primate. Pre-treatment with the OATP inhibitor rifampicin resulted in a pronounced decrease in [^{11}C]glyburide uptake by the liver (a, b). This resulted in a marked increase in [^{11}C]glyburide plasma concentrations (c) and the recovery of parent (unmetabolized) [^{11}C]glyburide in plasma (d). Radioactivity concentration is normalized to injected radioactivity amount per body weight and expressed as standardized uptake value (SUV). Adapted from Tournier et al., 2013.

Fig. 6. Serial abdominal PET images recorded in mice (a) and humans (b) after intravenous injection of a microdose of [^{11}C]erlotinib (scan 1) and after injection of a microdose of [^{11}C]erlotinib mixed with a pharmacologic dose of unlabeled erlotinib (10 mg/kg) (mice) or after pre-treatment with an oral therapeutic dose of erlotinib (300 mg) (humans) (scan 2). Anatomical structures are labeled with arrows (L, liver; GB, gall bladder; C, colon; D, duodenum; BD, bile duct). Radioactivity concentration is normalized to injected radioactivity amount per body weight and expressed as standardized uptake value (SUV). Concentration-time curves of [^{11}C]erlotinib in the liver of mice

(c) and humans (d) for scan 1 and scan 2. The effect of unlabeled erlotinib on liver distribution of [^{11}C]erlotinib is more pronounced in humans than in mice. Adapted from Bauer et al., 2017 and Traxl et al., 2015.

References

- Abbott, N. J., Patabendige, A. A. K., Dolman, D. E. M., Yusof, S. R., & Begley, D. J. (2010). Structure and function of the blood-brain barrier. *Neurobiology of Disease*, 37, 13–25.
- Agarwal, S., Hartz, A. M. S., Elmquist, W. F., & Bauer, B. (2011). Breast cancer resistance protein and P-glycoprotein in brain cancer: two gatekeepers team up. *Current Pharmaceutical Design*, 17, 2793–2802.
- Agrawal, M., Abraham, J., Balis, F. M., Edgerly, M., Stein, W. D., Bates, S., et al. (2003). Increased ^{99m}Tc-sestamibi accumulation in normal liver and drug-resistant tumors after the administration of the glycoprotein inhibitor, XR9576. *Clinical Cancer Research*, 9, 650–656.
- Ali, I., Slizgi, J. R., Kaullen, J. D., Ivanovic, M., Niemi, M., Stewart, P. W., et al. (2017). Transporter-Mediated Alterations in Patients With NASH Increase Systemic and Hepatic Exposure to an OATP and MRP2 Substrate. *Clinical Pharmacology and Therapeutics*. doi:10.1002/cpt.997
- Auvity, S., Chapy, H., Goutal, S., Caillé, F., Hosten, B., Smirnova, M., et al. (2017). Diphenhydramine as a selective probe to study H⁺-antiporter function at the blood-brain barrier: Application to [¹¹C]diphenhydramine positron emission tomography imaging. *Journal of Cerebral Blood Flow and Metabolism*, 37, 2185–2195.
- Awasthi, V., Pathuri, G., Agashe, H. B., & Gali, H. (2011). Synthesis and in vivo evaluation of p-¹⁸F-Fluorohippurate as a new radiopharmaceutical for assessment of renal function by PET. *Journal of Nuclear Medicine*, 52, 147–153.
- Bailey, D. L., & Willowson, K. P. (2013). An Evidence-Based Review of Quantitative SPECT Imaging and Potential Clinical Applications. *Journal of Nuclear Medicine*, 54, 83–89.
- Ballard, P., Yates, J. W. T., Yang, Z., Kim, D.-W., Yang, J. C.-H., Cantarini, M., et al. (2016). Preclinical Comparison of Osimertinib with Other EGFR-TKIs in EGFR-Mutant NSCLC Brain Metastases Models, and Early Evidence of Clinical Brain Metastases Activity. *Clinical Cancer Research*, 22, 5130–5140.
- Baltes, S., Gastens, A. M., Fedrowitz, M., Potschka, H., Kaefer, V., & Löscher, W. (2007). Differences in the transport of the antiepileptic drugs phenytoin, levetiracetam and carbamazepine by human and mouse P-glycoprotein. *Neuropharmacology*, 52, 333–346.
- Bankstahl, J. P., Bankstahl, M., Kuntner, C., Stanek, J., Wanek, T., Meier, M., et al. (2011). A novel positron emission tomography imaging protocol identifies seizure-induced regional overactivity of P-glycoprotein at the blood-brain barrier. *The Journal of Neuroscience*, 31, 8803–8811.
- Bartels, A. L., Kortekaas, R., Bart, J., Willemsen, A. T. M., de Klerk, O. L., de Vries, J. J., et al. (2009). Blood-brain barrier P-glycoprotein function decreases in specific brain regions with aging: a possible role in progressive neurodegeneration. *Neurobiology of Aging*, 30, 1818–1824.
- Bartels, A. L., Willemsen, A. T. M., Kortekaas, R., de Jong, B. M., de Vries, R., de Klerk, O., et al. (2008). Decreased blood-brain barrier P-glycoprotein function in the progression of Parkinson's disease, PSP and MSA. *Journal of Neural Transmission*, 115, 1001–1009.
- Bauer, M., Karch, R., Neumann, F., Abraham, A., Wagner, C. C., Kletter, K., et al. (2009). Age

- dependency of cerebral P-gp function measured with (R)-[11C]verapamil and PET. *European Journal of Clinical Pharmacology*, 65, 941–946.
- Bauer, M., Karch, R., Tournier, N., Cisternino, S., Wadsak, W., Hacker, M., et al. (2017a). Assessment of P-Glycoprotein Transport Activity at the Human Blood-Retina Barrier with (R)-11C-Verapamil PET. *Journal of Nuclear Medicine*, 58, 678–681.
- Bauer, M., Karch, R., Zeitlinger, M., Philippe, C., Römermann, K., Stanek, J., et al. (2015). Approaching complete inhibition of P-glycoprotein at the human blood-brain barrier: an (R)-[11C]verapamil PET study. *Journal of Cerebral Blood Flow and Metabolism*, 35, 743–746.
- Bauer, M., Matsuda, A., Wulkersdorfer, B., Philippe, C., Traxl, A., Özvegy-Laczka, C., et al. (2017b). Influence of OATPs on Hepatic Disposition of Erlotinib Measured With Positron Emission Tomography. *Clinical Pharmacology and Therapeutics*. doi:10.1002/cpt.888
- Bauer, M., Römermann, K., Karch, R., Wulkersdorfer, B., Stanek, J., Philippe, C., et al. (2016). Pilot PET Study to Assess the Functional Interplay Between ABCB1 and ABCG2 at the Human Blood-Brain Barrier. *Clinical Pharmacology and Therapeutics*, 100, 131–141.
- Bauer, M., Wulkersdorfer, B., Karch, R., Philippe, C., Jäger, W., Stanek, J., et al. (2017c). Effect of P-glycoprotein inhibition at the blood-brain barrier on brain distribution of (R)-[11C]verapamil in elderly vs. young subjects. *British Journal of Clinical Pharmacology*, 83, 1991–1999.
- Bauer, M., Zeitlinger, M., Karch, R., Matzneller, P., Stanek, J., Jäger, W., et al. (2012). Pgp-mediated interaction between (R)-[11C]verapamil and tariquidar at the human blood-brain barrier: a comparison with rat data. *Clinical Pharmacology and Therapeutics*, 91, 227–233.
- Benet, L. Z. (2009). The drug transporter-metabolism alliance: uncovering and defining the interplay. *Molecular Pharmaceutics*, 6, 1631–1643.
- Benveniste, H., Fowler, J. S., Rooney, W. D., Moller, D. H., Backus, W. W., Warner, D. A., et al. (2003). Maternal-fetal in vivo imaging: a combined PET and MRI study. *Journal of Nuclear Medicine*, 44, 1522–1530.
- Beyer, T., Hacker, M., & Goh, V. (2017). PET/MRI-knocking on the doors of the rich and famous. *The British Journal of Radiology*, 90, 20170347.
- Beyer, T., Townsend, D. W., Brun, T., Kinahan, P. E., Charron, M., Roddy, R., et al. (2000). A combined PET/CT scanner for clinical oncology. *Journal of Nuclear Medicine*, 41, 1369–1379.
- Brunner, M., Langer, O., Dobrozemsky, G., Müller, U., Zeitlinger, M., Mitterhauser, M., et al. (2004). [18F]Ciprofloxacin, a new positron emission tomography tracer for noninvasive assessment of the tissue distribution and pharmacokinetics of ciprofloxacin in humans. *Antimicrobial Agents and Chemotherapy*, 48, 3850–3857.
- Brunner, M., Langer, O., Sunder-Plassmann, R., Dobrozemsky, G., Müller, U., Wadsak, W., et al. (2005). Influence of functional haplotypes in the drug transporter gene ABCB1 on central nervous system drug distribution in humans. *Clinical Pharmacology and Therapeutics*, 78, 182–190.
- Burt, T., Rouse, D. C., Lee, K., Wu, H., Layton, A. T., Hawk, T. C., et al. (2015). Intraarterial Microdosing: A Novel Drug Development Approach, Proof-of-Concept PET Study in Rats. *Journal of Nuclear Medicine*, 56, 1793–1799.

- Camidge, D. R., Pao, W., & Sequist, L. V. (2014). Acquired resistance to TKIs in solid tumours: learning from lung cancer. *Nature Reviews. Clinical Oncology*, 11, 473–481.
- Cattelotte, J., Tournier, N., Rizzo-Padoin, N., Schinkel, A. H., Scherrmann, J.-M., & Cisternino, S. (2009). Changes in dipole membrane potential at the mouse blood-brain barrier enhance the transport of 99mTechnetium Sestamibi more than inhibiting Abcb1, Abcc1, or Abcg2. *Journal of Neurochemistry*, 108, 767–775.
- Chapy, H., Saubaméa, B., Tournier, N., Bourasset, F., Behar-Cohen, F., Declèves, X., et al. (2016). Blood-brain and retinal barriers show dissimilar ABC transporter impacts and concealed effect of P-glycoprotein on a novel verapamil influx carrier. *British Journal of Pharmacology*, 173, 497–510.
- Chen, C. C., Meadows, B., Regis, J., Kalafsky, G., Fojo, T., Carrasquillo, J. A., et al. (1997). Detection of in vivo P-glycoprotein inhibition by PSC 833 using Tc-99m sestamibi. *Clinical Cancer Research*, 3, 545–552.
- Choo, E. F., & Salphati, L. (2018). Leveraging Humanized Animal Models to Understand Human Drug Disposition: Opportunities, Challenges, and Future Directions. *Clinical Pharmacology and Therapeutics*, 103, 188–192.
- Chu, X., Korzekwa, K., Elsbey, R., Fenner, K., Galetin, A., Lai, Y., et al. (2013). Intracellular Drug Concentrations and Transporters: Measurement, Modeling, and Implications for the Liver. *Clinical Pharmacology and Therapeutics*, 94, 126–141.
- Collier, T. L., Maresca, K. P., Normandin, M. D., Richardson, P., McCarthy, T. J., Liang, S. H., et al. (2017a). Brain Penetration of the ROS1/ALK Inhibitor Lorlatinib Confirmed by PET. *Molecular Imaging*, 16. doi:10.1177/1536012117736669
- Collier, T. L., Normandin, M. D., Stephenson, N. A., Livni, E., Liang, S. H., Wooten, D. W., et al. (2017b). Synthesis and preliminary PET imaging of 11C and 18F isotopologues of the ROS1/ALK inhibitor lorlatinib. *Nature Communications*, 8, 15761.
- Cunha, L., Szigeti, K., Mathé, D., & Metello, L. F. (2014). The role of molecular imaging in modern drug development. *Drug Discovery Today*, 19, 936–948.
- Custodio, J. M., Wu, C.-Y., & Benet, L. Z. (2008). Predicting drug disposition, absorption/elimination/transporter interplay and the role of food on drug absorption. *Advanced Drug Delivery Reviews*, 60, 717–733.
- Damont, A., Goutal, S., Auvity, S., Valette, H., Kuhnast, B., Saba, W., et al. (2016). Imaging the impact of cyclosporin A and dipyridamole on P-glycoprotein (ABCB1) function at the blood-brain barrier: A [(11)C]-N-desmethyl-loperamide PET study in nonhuman primates. *European Journal of Pharmaceutical Sciences*, 91, 98–104.
- Davies, B., & Morris, T. (1993). Physiological parameters in laboratory animals and humans. *Pharmaceutical Research*, 10, 1093–1095.
- de Graaf, W., Häusler, S., Heger, M., van Ginhoven, T. M., van Cappellen, G., Bennink, R. J., et al. (2011). Transporters involved in the hepatic uptake of (99m)Tc-mebrofenin and indocyanine green. *Journal of Hepatology*, 54, 738–745.
- de Klerk, O. L., Willemsen, A. T. M., Bosker, F. J., Bartels, A. L., Hendrikse, N. H., den Boer, J. A., et al. (2010). Regional increase in P-glycoprotein function in the blood-brain barrier of

- patients with chronic schizophrenia: a PET study with [(11)C]verapamil as a probe for P-glycoprotein function. *Psychiatry Research*, 183, 151–156.
- de Klerk, O. L., Willemsen, A. T. M., Roosink, M., Bartels, A. L., Hendrikse, N. H., Bosker, F. J., et al. (2009). Locally increased P-glycoprotein function in major depression: a PET study with [11C]verapamil as a probe for P-glycoprotein function in the blood-brain barrier. *The International Journal of Neuropsychopharmacology*, 12, 895–904.
- De Lombaerde, S., Neyt, S., Kersemans, K., Verhoeven, J., Devisscher, L., Van Vlierberghe, H., et al. (2017). Synthesis, in vitro and in vivo evaluation of 3 β -[18F]fluorocholeic acid for the detection of drug-induced cholestasis in mice. *PloS One*, 12, e0173529.
- Deo, A. K., Borson, S., Link, J. M., Domino, K., Eary, J. F., Ke, B., et al. (2014). Activity of P-Glycoprotein, a β -Amyloid Transporter at the Blood-Brain Barrier, Is Compromised in Patients with Mild Alzheimer Disease. *Journal of Nuclear Medicine*, 55, 1106–1111.
- Derebek, E., Kirkali, Z., Dogan, A. S., Degirmenci, B., Yilmaz, M., Igci, E., et al. (1996). 99mTc-MIBI scintigraphy in metastatic renal cell carcinoma: clinical validation of the relationship between 99mTc-MIBI uptake and P-glycoprotein expression in tumour tissue. *European Journal of Nuclear Medicine*, 23, 976–979.
- Doi, N., Tomiyama, Y., Kawase, T., Nishina, S., Yoshioka, N., Hara, Y., et al. (2011). Focal nodular hyperplasia-like nodule with reduced expression of organic anion transporter 1B3 in alcoholic liver cirrhosis. *Internal Medicine*, 50, 1193–1199.
- Dolovich, M., & Labiris, R. (2004). Imaging drug delivery and drug responses in the lung. *Proceedings of the American Thoracic Society*, 1, 329–337.
- Durmus, S., Hendrikx, J. J. M. A., & Schinkel, A. H. (2015). Apical ABC transporters and cancer chemotherapeutic drug disposition. *Advances in Cancer Research*, 125, 1–41.
- Durmus, S., van Hoppe, S., & Schinkel, A. H. (2016). The impact of Organic Anion-Transporting Polypeptides (OATPs) on disposition and toxicity of antitumor drugs: Insights from knockout and humanized mice. *Drug Resistance Updates*, 27, 72–88.
- Eary, J. F., Link, J. M., Muzi, M., Conrad, E. U., Mankoff, D. A., White, J. K., et al. (2011). Multiagent PET for risk characterization in sarcoma. *Journal of Nuclear Medicine*, 52, 541–546.
- Ekins, S., Polli, J. E., Swaan, P. W., & Wright, S. H. (2012). Computational Modeling to Accelerate the Identification of Substrates and Inhibitors For Transporters That Affect Drug Disposition. *Clinical Pharmacology and Therapeutics*, 92, 661–665.
- El-Gebali, S., Bentz, S., Hediger, M. A., & Anderle, P. (2013). Solute carriers (SLCs) in cancer. *Molecular Aspects of Medicine*, 34, 719–734.
- EMA. (2013). Guideline on the investigation of drug interactions. Retrieved from http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2012/07/WC500129606.pdf
- Eyal, S., Chung, F. S., Muzi, M., Link, J. M., Mankoff, D. A., Kaddoumi, A., et al. (2009a). Simultaneous PET imaging of P-glycoprotein inhibition in multiple tissues in the pregnant nonhuman primate. *Journal of Nuclear Medicine*, 50, 798–806.

- Eyal, S., Hsiao, P., & Unadkat, J. D. (2009b). Drug interactions at the blood-brain barrier: fact or fantasy? *Pharmacology & Therapeutics*, 123, 80–104.
- Fan, J., & de Lannoy, I. A. M. (2014). Pharmacokinetics. *Biochemical Pharmacology*, 87, 93–120.
- Fattinger, K., Cattori, V., Hagenbuch, B., Meier, P. J., & Stieger, B. (2000). Rifamycin SV and rifampicin exhibit differential inhibition of the hepatic rat organic anion transporting polypeptides, Oatp1 and Oatp2. *Hepatology*, 32, 82–86.
- Fattinger, K., Funk, C., Pantze, M., Weber, C., Reichen, J., Stieger, B., et al. (2001). The endothelin antagonist bosentan inhibits the canalicular bile salt export pump: a potential mechanism for hepatic adverse reactions. *Clinical Pharmacology and Therapeutics*, 69, 223–231.
- FDA. (2017). Clinical Drug Interaction Studies - Study Design, Data Analysis, and Clinical Implications - Guidance for Industry. Retrieved from <https://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM292362.pdf>
- Feldmann, M., Asselin, M.-C., Liu, J., Wang, S., McMahon, A., Anton-Rodriguez, J., et al. (2013). P-glycoprotein expression and function in patients with temporal lobe epilepsy: a case-control study. *Lancet Neurology*, 12, 777–785.
- Frey, E. C., Humm, J. L., & Ljungberg, M. (2012). Accuracy and precision of radioactivity quantification in nuclear medicine images. *Seminars in Nuclear Medicine*, 42, 208–218.
- Frisch, K., Jakobsen, S., Sørensen, M., Munk, O. L., Alstrup, A. K. O., Ott, P., et al. (2012). [N-methyl-11C]cholylsarcosine, a novel bile acid tracer for PET/CT of hepatic excretory function: radiosynthesis and proof-of-concept studies in pigs. *Journal of Nuclear Medicine*, 53, 772–778.
- Frisch, K., & Sørensen, M. (2014). On fluoro-18 labeling of bile acids. *Nuclear Medicine and Biology*, 41, 775.
- Fung, H.-L., & Jusko, W. J. (2015). Perspectives on the history and scientific contributions of Gerhard Levy. *Journal of Pharmacokinetics and Pharmacodynamics*, 42, 429–446.
- Funk, C., Pantze, M., Jehle, L., Ponelle, C., Scheuermann, G., Lazendic, M., et al. (2001). Troglitazone-induced intrahepatic cholestasis by an interference with the hepatobiliary export of bile acids in male and female rats. Correlation with the gender difference in troglitazone sulfate formation and the inhibition of the canalicular bile salt export pump (Bsep) by troglitazone and troglitazone sulfate. *Toxicology*, 167, 83–98.
- Galante, E., Okamura, T., Sander, K., Kikuchi, T., Okada, M., Zhang, M.-R., et al. (2014). Development of purine-derived 18F-labeled pro-drug tracers for imaging of MRP1 activity with PET. *Journal of Medicinal Chemistry*, 57, 1023–1032.
- Georgiou, L., Penny, J., Nicholls, G., Woodhouse, N., Blé, F.-X., Hubbard Cristinacce, P. L., et al. (2017). Quantitative Assessment of Liver Function Using Gadoxetate-Enhanced Magnetic Resonance Imaging. *Investigative Radiology*, 52, 111–119.
- Ghibellini, G., Leslie, E. M., Pollack, G. M., & Brouwer, K. L. R. (2008). Use of tc-99m mebrofenin as a clinical probe to assess altered hepatobiliary transport: integration of in vitro, pharmacokinetic modeling, and simulation studies. *Pharmaceutical Research*, 25, 1851–

1860.

- Giacomini, K. M., Huang, S.-M., Tweedie, D. J., Benet, L. Z., Brouwer, K. L. R., Chu, X., et al. (2010). Membrane transporters in drug development. *Nature Reviews. Drug Discovery*, 9, 215–236.
- Gui, C., Obaidat, A., Chaguturu, R., & Hagenbuch, B. (2010). Development of a cell-based high-throughput assay to screen for inhibitors of organic anion transporting polypeptides 1B1 and 1B3. *Current Chemical Genomics*, 4, 1–8.
- Gustafsson, S., Eriksson, J., Syvänen, S., Eriksson, O., Hammarlund-Udenaes, M., & Antoni, G. (2017). Combined PET and microdialysis for in vivo estimation of drug blood-brain barrier transport and brain unbound concentrations. *NeuroImage*, 155, 177–186.
- Hagenbuch, B., & Stieger, B. (2013). The SLCO (former SLC21) superfamily of transporters. *Molecular Aspects of Medicine*, 34, 396–412.
- Hediger, M. A., Clémenton, B., Burrier, R. E., & Bruford, E. A. (2013). The ABCs of membrane transporters in health and disease (SLC series): introduction. *Molecular Aspects of Medicine*, 34, 95–107.
- Hendrikse, N. H., & Vaalburg, W. (2002). Dynamics of multidrug resistance: P-glycoprotein analyses with positron emission tomography. *Methods*, 27, 228–233.
- Hillgren, K. M., Keppler, D., Zur, A. A., Giacomini, K. M., Stieger, B., Cass, C. E., et al. (2013). Emerging transporters of clinical importance: an update from the International Transporter Consortium. *Clinical Pharmacology and Therapeutics*, 94, 52–63.
- Hillyar, C. R. T., Knight, J. C., Vallis, K. A., & Cornelissen, B. (2015). PET and SPECT Imaging for the Acceleration of Anti-Cancer Drug Development. *Current Drug Targets*, 16, 582–591.
- Holohan, C., Van Schaeybroeck, S., Longley, D. B., & Johnston, P. G. (2013). Cancer drug resistance: an evolving paradigm. *Nature Reviews. Cancer*, 13, 714–726.
- Hosoya, K., Tomi, M., & Tachikawa, M. (2011). Strategies for therapy of retinal diseases using systemic drug delivery: relevance of transporters at the blood-retinal barrier. *Expert Opinion on Drug Delivery*, 8, 1571–1587.
- Hosten, B., Boisgard, R., Jacob, A., Goutal, S., Saubaméa, B., Dollé, F., et al. (2013). [¹¹C]befloxatone brain kinetics is not influenced by Bcrp function at the blood-brain barrier: a PET study using Bcrp TGEM knockout rats. *European Journal of Pharmaceutical Sciences*, 50, 520–525.
- Hsiao, P., Sasongko, L., Link, J. M., Mankoff, D. A., Muzi, M., Collier, A. C., et al. (2006). Verapamil P-glycoprotein transport across the rat blood-brain barrier: cyclosporine, a concentration inhibition analysis, and comparison with human data. *The Journal of Pharmacology and Experimental Therapeutics*, 317, 704–710.
- Hume, W. E., Shingaki, T., Takashima, T., Hashizume, Y., Okauchi, T., Katayama, Y., et al. (2013). The synthesis and biodistribution of [¹¹C]metformin as a PET probe to study hepatobiliary transport mediated by the multi-drug and toxin extrusion transporter 1 (MATE1) in vivo. *Bioorganic & Medicinal Chemistry*, 21, 7584–7590.
- Huppertz, A., Breuer, J., Fels, L. M., Schultze-Mosgau, M., Sutter, G., Klein, S., et al. (2011).

Evaluation of possible drug-drug interaction between gadoxetic acid and erythromycin as an inhibitor of organic anion transporting peptides (OATP). *Journal of Magnetic Resonance Imaging: JMIR*, 33, 409–416.

ICH Expert Working Group. (2009). *Guidance on nonclinical safety studies for the conduct of human clinical trials and marketing authorization for pharmaceuticals M3(R2)* (No. <http://www.ich.org/products/guidelines/safety/safety-single/article/guidance-on-nonclinical-safety-studies-for-the-conduct-of-human-clinical-trials-and-marketing-author.html>).

Ieiri, I. (2012). Functional significance of genetic polymorphisms in P-glycoprotein (MDR1, ABCB1) and breast cancer resistance protein (BCRP, ABCG2). *Drug Metabolism and Pharmacokinetics*, 27, 85–105.

Ikoma, Y., Takano, A., Ito, H., Kusuhara, H., Sugiyama, Y., Arakawa, R., et al. (2006). Quantitative analysis of ¹¹C-verapamil transfer at the human blood-brain barrier for evaluation of P-glycoprotein function. *Journal of Nuclear Medicine*, 47, 1531–1537.

Ingrisch, M., & Sourbron, S. (2013). Tracer-kinetic modeling of dynamic contrast-enhanced MRI and CT: a primer. *Journal of Pharmacokinetics and Pharmacodynamics*, 40, 281–300.

Innis, R. B., Cunningham, V. J., Delforge, J., Fujita, M., Gjedde, A., Gunn, R. N., et al. (2007). Consensus nomenclature for in vivo imaging of reversibly binding radioligands. *Journal of Cerebral Blood Flow and Metabolism*, 27, 1533–1539.

Ito, K., Uchida, Y., Ohtsuki, S., Aizawa, S., Kawakami, H., Katsukura, Y., et al. (2011). Quantitative membrane protein expression at the blood-brain barrier of adult and younger cynomolgus monkeys. *Journal of Pharmaceutical Sciences*, 100, 3939–3950.

Jakobsen, S., Busk, M., Jensen, J. B., Munk, O. L., Zois, N. E., Alstrup, A. K. O., et al. (2016). A PET Tracer for Renal Organic Cation Transporters, ¹¹C-Metformin: Radiosynthesis and Preclinical Proof-of-Concept Studies. *Journal of Nuclear Medicine*, 57, 615–621.

Jensen, J. B., Sundelin, E. I., Jakobsen, S., Gormsen, L. C., Munk, O. L., Frøkiær, J., et al. (2016). [¹¹C]-Labeled Metformin Distribution in the Liver and Small Intestine Using Dynamic Positron Emission Tomography in Mice Demonstrates Tissue-Specific Transporter Dependency. *Diabetes*, 65, 1724–1730.

Jia, J., Puls, D., Oswald, S., Jedlitschky, G., Kühn, J. P., Weitschies, W., et al. (2014a). Characterization of the intestinal and hepatic uptake/efflux transport of the magnetic resonance imaging contrast agent gadolinium-ethoxylbenzyl-diethylenetriamine-pentaacetic acid. *Investigative Radiology*, 49, 78–86.

Jia, L., Jiang, D., Hu, P., Li, X., Shi, H., Cheng, D., et al. (2014b). Synthesis and evaluation of (¹⁸F)-labeled bile acid compound: a potential PET imaging agent for FXR-related diseases. *Nuclear Medicine and Biology*, 41, 495–500.

Kalvass, J. C., Polli, J. W., Bourdet, D. L., Feng, B., Huang, S.-M., Liu, X., et al. (2013). Why clinical modulation of efflux transport at the human blood-brain barrier is unlikely: the ITC evidence-based position. *Clinical Pharmacology and Therapeutics*, 94, 80–94.

Kaneko, K.-I., Tanaka, M., Ishii, A., Katayama, Y., Nakaoka, T., Irie, S., et al. (2018). A clinical quantitative evaluation of hepatobiliary transport of [¹¹C]Dehydropravastatin in humans using positron emission tomography. *Drug Metabolism and Disposition*.

doi:10.1124/dmd.118.080408

- Kannan, P., Brimacombe, K. R., Zoghbi, S. S., Liow, J.-S., Morse, C., Taku, A. K., et al. (2010). N-desmethyl-loperamide is selective for P-glycoprotein among three ATP-binding cassette transporters at the blood-brain barrier. *Drug Metabolism and Disposition*, 38, 917–922.
- Kannan, P., John, C., Zoghbi, S. S., Halldin, C., Gottesman, M. M., Innis, R. B., et al. (2009). Imaging the function of P-glycoprotein with radiotracers: pharmacokinetics and in vivo applications. *Clinical Pharmacology and Therapeutics*, 86, 368–377.
- Kao, C. H., Hsieh, J. F., Tsai, S. C., Ho, Y. J., Changlai, S. P., & Lee, J. K. (2001). Paclitaxel-Based chemotherapy for non-small cell lung cancer: predicting the response with 99mTc-tetrofosmin chest imaging. *Journal of Nuclear Medicine*, 42, 17–20.
- Kato, N., Yokawa, T., Tamura, A., Heshiki, A., Ebert, W., & Weinmann, H.-J. (2002). Gadolinium-ethoxybenzyl-diethylenetriamine-pentaacetic acid interaction with clinical drugs in rats. *Investigative Radiology*, 37, 680–684.
- Ke, A. B., Eyal, S., Chung, F. S., Link, J. M., Mankoff, D. A., Muzi, M., et al. (2013). Modeling cyclosporine A inhibition of the distribution of a P-glycoprotein PET ligand, 11C-verapamil, into the maternal brain and fetal liver of the pregnant nonhuman primate: impact of tissue blood flow and site of inhibition. *Journal of Nuclear Medicine*, 54, 437–446.
- Kell, D. B. (2015). What would be the observable consequences if phospholipid bilayer diffusion of drugs into cells is negligible? *Trends in Pharmacological Sciences*, 36, 15–21.
- Keskitalo, J. E., Zolk, O., Fromm, M. F., Kurkinen, K. J., Neuvonen, P. J., & Niemi, M. (2009). ABCG2 polymorphism markedly affects the pharmacokinetics of atorvastatin and rosuvastatin. *Clinical Pharmacology and Therapeutics*, 86, 197–203.
- Kilbourn, M. R. (2017). Small Molecule PET Tracers for Transporter Imaging. *Seminars in Nuclear Medicine*, 47, 536–552.
- Kim, I.-W., Booth-Genthe, C., & Ambudkar, S. V. (2008). Relationship between drugs and functional activity of various mammalian P-glycoproteins (ABCB1). *Mini Reviews in Medicinal Chemistry*, 8, 193–200.
- Kitao, A., Matsui, O., Yoneda, N., Kozaka, K., Shinmura, R., Koda, W., et al. (2011). The uptake transporter OATP8 expression decreases during multistep hepatocarcinogenesis: correlation with gadoxetic acid enhanced MR imaging. *European Radiology*, 21, 2056–2066.
- Kitao, A., Zen, Y., Matsui, O., Gabata, T., Kobayashi, S., Koda, W., et al. (2010). Hepatocellular carcinoma: signal intensity at gadoxetic acid-enhanced MR Imaging--correlation with molecular transporters and histopathologic features. *Radiology*, 256, 817–826.
- Kobayashi, D., Ieiri, I., Hirota, T., Takane, H., Maegawa, S., Kigawa, J., et al. (2005). Functional assessment of ABCG2 (BCRP) gene polymorphisms to protein expression in human placenta. *Drug Metabolism and Disposition*, 33, 94–101.
- König, J., Müller, F., & Fromm, M. F. (2013). Transporters and Drug-Drug Interactions: Important Determinants of Drug Disposition and Effects. *Pharmacological Reviews*, 65, 944–966.
- König, J., Seithel, A., Gradhand, U., & Fromm, M. F. (2006). Pharmacogenomics of human OATP transporters. *Naunyn-Schmiedeberg's Archives of Pharmacology*, 372, 432–443.

- Kortekaas, R., Leenders, K. L., van Oostrom, J. C. H., Vaalburg, W., Bart, J., Willemsen, A. T. M., et al. (2005). Blood-brain barrier dysfunction in parkinsonian midbrain in vivo. *Annals of Neurology*, 57, 176–179.
- Kreisl, W. C., Bhatia, R., Morse, C. L., Woock, A. E., Zoghbi, S. S., Shetty, H. U., et al. (2015). Increased permeability-glycoprotein inhibition at the human blood-brain barrier can be safely achieved by performing PET during peak plasma concentrations of tariquidar. *Journal of Nuclear Medicine*, 56, 82–87.
- Kreisl, W. C., Liow, J.-S., Kimura, N., Seneca, N., Zoghbi, S. S., Morse, C. L., et al. (2010). P-glycoprotein function at the blood-brain barrier in humans can be quantified with the substrate radiotracer ¹¹C-N-desmethyl-loperamide. *Journal of Nuclear Medicine*, 51, 559–566.
- Kuntner, C., Bankstahl, J. P., Bankstahl, M., Stanek, J., Wanek, T., Stundner, G., et al. (2010). Dose-response assessment of tariquidar and elacridar and regional quantification of P-glycoprotein inhibition at the rat blood-brain barrier using (R)-[(¹¹C)]verapamil PET. *European Journal of Nuclear Medicine and Molecular Imaging*, 37, 942–953.
- Kurdziel, K. A., Kalen, J. D., Hirsch, J. I., Wilson, J. D., Bear, H. D., Logan, J., et al. (2011). Human dosimetry and preliminary tumor distribution of ¹⁸F-fluoropaclitaxel in healthy volunteers and newly diagnosed breast cancer patients using PET/CT. *Journal of Nuclear Medicine*, 52, 1339–1345.
- Kusuhara, H. (2013). Imaging in the study of membrane transporters. *Clinical Pharmacology and Therapeutics*, 94, 33–36.
- Kusuhara, H., & Sugiyama, Y. (2009). In vitro-in vivo extrapolation of transporter-mediated clearance in the liver and kidney. *Drug Metabolism and Pharmacokinetics*, 24, 37–52.
- Lagadec, M., Doblas, S., Giraudeau, C., Ronot, M., Lambert, S. A., Fasseu, M., et al. (2015). Advanced fibrosis: Correlation between pharmacokinetic parameters at dynamic gadoxetate-enhanced MR imaging and hepatocyte organic anion transporter expression in rat liver. *Radiology*, 274, 379–386.
- Langer, O. (2016). Use of PET Imaging to Evaluate Transporter-Mediated Drug-Drug Interactions. *Journal of Clinical Pharmacology*, 56 Suppl 7, S143-156.
- Langer, O., Bauer, M., Hammers, A., Karch, R., Pataria, E., Koepp, M. J., et al. (2007). Pharmacoresistance in epilepsy: a pilot PET study with the P-glycoprotein substrate R-[(¹¹C)]verapamil. *Epilepsia*, 48, 1774–1784.
- Lazarova, N., Zoghbi, S. S., Hong, J., Seneca, N., Tuan, E., Gladding, R. L., et al. (2008). Synthesis and evaluation of [N-methyl-¹¹C]N-desmethyl-loperamide as a new and improved PET radiotracer for imaging P-gp function. *Journal of Medicinal Chemistry*, 51, 6034–6043.
- Lee, S.-C., Arya, V., Yang, X., Volpe, D. A., & Zhang, L. (2017). Evaluation of transporters in drug development: Current status and contemporary issues. *Advanced Drug Delivery Reviews*, 116, 100–118.
- Leonhardt, M., Keiser, M., Oswald, S., Kühn, J., Jia, J., Grube, M., et al. (2010). Hepatic Uptake of the Magnetic Resonance Imaging Contrast Agent Gd-EOB-DTPA: Role of Human Organic Anion Transporters. *Drug Metabolism and Disposition*, 38, 1024–1028.
- Levy, G. (1966). Kinetics of pharmacologic effects. *Clinical Pharmacology and Therapeutics*, 7,

362–372.

- Li, M., Yuan, H., Li, N., Song, G., Zheng, Y., Baratta, M., et al. (2008). Identification of interspecies difference in efflux transporters of hepatocytes from dog, rat, monkey and human. *European Journal of Pharmaceutical Sciences*, 35, 114–126.
- Li, Z., & Conti, P. S. (2010). Radiopharmaceutical chemistry for positron emission tomography. *Advanced Drug Delivery Reviews*, 62, 1031–1051.
- Ling, J., Johnson, K. A., Miao, Z., Rakhit, A., Pantze, M. P., Hamilton, M., et al. (2006). Metabolism and excretion of erlotinib, a small molecule inhibitor of epidermal growth factor receptor tyrosine kinase, in healthy male volunteers. *Drug Metabolism and Disposition*, 34, 420–426.
- Liow, J.-S., Kreisl, W., Zoghbi, S. S., Lazarova, N., Seneca, N., Gladding, R. L., et al. (2009). P-glycoprotein function at the blood-brain barrier imaged using ¹¹C-N-desmethyl-loperamide in monkeys. *Journal of Nuclear Medicine*, 50, 108–115.
- Liu, L., Collier, A. C., Link, J. M., Domino, K. B., Mankoff, D. A., Eary, J. F., et al. (2015). Modulation of P-glycoprotein at the Human Blood-Brain Barrier by Quinidine or Rifampin Treatment: A Positron Emission Tomography Imaging Study. *Drug Metabolism and Disposition*, 43, 1795–1804.
- Lowenstein, J., & Grantham, J. J. (2016). The rebirth of interest in renal tubular function. *American Journal of Physiology. Renal Physiology*, 310, F1351–1355.
- Luker, G. D., Fracasso, P. M., Dobkin, J., & Piwnica-Worms, D. (1997). Modulation of the multidrug resistance P-glycoprotein: detection with technetium-99m-sestamibi in vivo. *Journal of Nuclear Medicine*, 38, 369–372.
- Luna-Tortós, C., Fedrowitz, M., & Löscher, W. (2008). Several major antiepileptic drugs are substrates for human P-glycoprotein. *Neuropharmacology*, 55, 1364–1375.
- Luurtsema, G., Elsinga, P., Dierckx, R., Boellaard, R., & van Waarde, A. (2016). PET Tracers for Imaging of ABC Transporters at the Blood-Brain Barrier: Principles and Strategies. *Current Pharmaceutical Design*, 22, 5779–5785.
- Luurtsema, G., Molthoff, C. F. M., Schuit, R. C., Windhorst, A. D., Lammertsma, A. A., & Franssen, E. J. F. (2005). Evaluation of (R)-[¹¹C]verapamil as PET tracer of P-glycoprotein function in the blood-brain barrier: kinetics and metabolism in the rat. *Nuclear Medicine and Biology*, 32, 87–93.
- Luurtsema, G., Molthoff, C. F. M., Windhorst, A. D., Smit, J. W., Keizer, H., Boellaard, R., et al. (2003). (R)- and (S)-[¹¹C]verapamil as PET-tracers for measuring P-glycoprotein function: in vitro and in vivo evaluation. *Nuclear Medicine and Biology*, 30, 747–751.
- Maeda, K., & Sugiyama, Y. (2008). Impact of genetic polymorphisms of transporters on the pharmacokinetic, pharmacodynamic and toxicological properties of anionic drugs. *Drug Metabolism and Pharmacokinetics*, 23, 223–235.
- Mairinger, S., Erker, T., Muller, M., & Langer, O. (2011). PET and SPECT radiotracers to assess function and expression of ABC transporters in vivo. *Current Drug Metabolism*, 12, 774–792.
- Mairinger, S., Langer, O., Kuntner, C., Wanek, T., Bankstahl, J. P., Bankstahl, M., et al. (2010). Synthesis and in vivo evaluation of the putative breast cancer resistance protein inhibitor

[11C]methyl 4-((4-(2-(6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolin-2-yl)ethyl)phenyl)amino-carbonyl)-2-(quinoline-2-carbonylamino)benzoate. *Nuclear Medicine and Biology*, 37, 637–644.

Mann, A., Han, H., & Eyal, S. (2016). Imaging transporters: Transforming diagnostic and therapeutic development. *Clinical Pharmacology and Therapeutics*, 100, 479–488.

Mann, A., Semenenko, I., Meir, M., & Eyal, S. (2015). Molecular Imaging of Membrane Transporters' Activity in Cancer: a Picture is Worth a Thousand Tubes. *The AAPS Journal*, 17, 788–801.

Mannermaa, E., Vellonen, K.-S., & Urtti, A. (2006). Drug transport in corneal epithelium and blood-retina barrier: emerging role of transporters in ocular pharmacokinetics. *Advanced Drug Delivery Reviews*, 58, 1136–1163.

Manohar, S., & Leung, N. (2017). Cisplatin nephrotoxicity: a review of the literature. *Journal of Nephrology*. doi:10.1007/s40620-017-0392-z

Marie, S., Cisternino, S., Buvat, I., Declèves, X., & Tournier, N. (2017). Imaging Probes and Modalities for the Study of Solute Carrier O (SLCO)-Transport Function In Vivo. *Journal of Pharmaceutical Sciences*, 106, 2335–2344.

Martignoni, M., Groothuis, G. M. M., & de Kanter, R. (2006). Species differences between mouse, rat, dog, monkey and human CYP-mediated drug metabolism, inhibition and induction. *Expert Opinion on Drug Metabolism & Toxicology*, 2, 875–894.

Matsson, P., Pedersen, J. M., Norinder, U., Bergström, C. A. S., & Artursson, P. (2009). Identification of novel specific and general inhibitors of the three major human ATP-binding cassette transporters P-gp, BCRP and MRP2 among registered drugs. *Pharmaceutical Research*, 26, 1816–1831.

Matsuda, A., Karch, R., Bauer, M., Traxl, A., Zeitlinger, M., & Langer, O. (2017). A Prediction Method for P-glycoprotein-Mediated Drug-Drug Interactions at the Human Blood-Brain Barrier From Blood Concentration-Time Profiles, Validated With PET Data. *Journal of Pharmaceutical Sciences*, 106, 2780–2786.

Miura, T., Ban, D., Tanaka, S., Mogushi, K., Kudo, A., Matsumura, S., et al. (2015). Distinct clinicopathological phenotype of hepatocellular carcinoma with ethoxybenzyl-magnetic resonance imaging hyperintensity: association with gene expression signature. *American Journal of Surgery*, 210, 561–569.

Mohan, H. K., Routledge, T., Cane, P., Livieratos, L., Ballinger, J. R., & Peters, A. M. (2016). Does the Clearance of Inhaled (99m)Tc-Sestamibi Correlate with Multidrug Resistance Protein 1 Expression in the Human Lung? *Radiology*, 280, 924–930.

Muzi, M., Mankoff, D. A., Link, J. M., Shoner, S., Collier, A. C., Sasongko, L., et al. (2009). Imaging of cyclosporine inhibition of P-glycoprotein activity using 11C-verapamil in the brain: studies of healthy humans. *Journal of Nuclear Medicine*, 50, 1267–1275.

Nassif, A., Jia, J., Keiser, M., Oswald, S., Modess, C., Nagel, S., et al. (2012). Visualization of hepatic uptake transporter function in healthy subjects by using gadoteric acid-enhanced MR imaging. *Radiology*, 264, 741–750.

Neyt, S., Huisman, M. T., Vanhove, C., De Man, H., Vliegen, M., Moerman, L., et al. (2013). In

- vivo visualization and quantification of (Disturbed) Oatp-mediated hepatic uptake and Mrp2-mediated biliary excretion of ^{99m}Tc-mebrofenin in mice. *Journal of Nuclear Medicine*, 54, 624–630.
- Nickel, S., Clerkin, C. G., Selo, M. A., & Ehrhardt, C. (2016). Transport mechanisms at the pulmonary mucosa: implications for drug delivery. *Expert Opinion on Drug Delivery*, 13, 667–690.
- Niemi, M., Pasanen, M. K., & Neuvonen, P. J. (2011). Organic anion transporting polypeptide 1B1: a genetically polymorphic transporter of major importance for hepatic drug uptake. *Pharmacological Reviews*, 63, 157–181.
- Nishimura, M., & Naito, S. (2005). Tissue-specific mRNA expression profiles of human ATP-binding cassette and solute carrier transporter superfamilies. *Drug Metabolism and Pharmacokinetics*, 20, 452–477.
- Nishimura, M., & Naito, S. (2008). Tissue-specific mRNA expression profiles of human solute carrier transporter superfamilies. *Drug Metabolism and Pharmacokinetics*, 23, 22–44.
- Ohtsuki, S., Uchida, Y., Kubo, Y., & Terasaki, T. (2011). Quantitative targeted absolute proteomics-based ADME research as a new path to drug discovery and development: methodology, advantages, strategy, and prospects. *Journal of Pharmaceutical Sciences*, 100, 3547–3559.
- Okamura, T., Kikuchi, T., Okada, M., Toramatsu, C., Fukushi, K., Takei, M., et al. (2009). Noninvasive and quantitative assessment of the function of multidrug resistance-associated protein 1 in the living brain. *Journal of Cerebral Blood Flow and Metabolism*, 29, 504–511.
- Okamura, T., Kikuchi, T., Okada, M., Wakizaka, H., & Zhang, M.-R. (2013). Imaging of activity of multidrug resistance-associated protein 1 in the lungs. *American Journal of Respiratory Cell and Molecular Biology*, 49, 335–340.
- Okubo, H., Ando, H., Kokubu, S., Miyazaki, A., Watanabe, S., & Fujimura, A. (2013). Polymorphisms in the organic anion transporting polypeptide genes influence liver parenchymal enhancement in gadoteric acid-enhanced MRI. *Pharmacogenomics*, 14, 1573–1582.
- Ørntoft, N. W., Munk, O. L., Frisch, K., Ott, P., Keiding, S., & Sørensen, M. (2017). Hepatobiliary transport kinetics of the conjugated bile acid tracer ¹¹C-CSar quantified in healthy humans and patients by positron emission tomography. *Journal of Hepatology*, 67, 321–327.
- Pastor, C. M., Müllhaupt, B., & Stieger, B. (2014). The role of organic anion transporters in diagnosing liver diseases by magnetic resonance imaging. *Drug Metabolism and Disposition*, 42, 675–684.
- Patilea-Vrana, G., & Unadkat, J. D. (2016). Transport vs. Metabolism: What Determines the Pharmacokinetics and Pharmacodynamics of Drugs? Insights From the Extended Clearance Model. *Clinical Pharmacology and Therapeutics*, 100, 413–418.
- Pedersen, L., Jensen, J. B., Wogensen, L., Munk, O. L., Jessen, N., Frøkiær, J., et al. (2016). Renal PET-imaging with (¹¹)C-metformin in a transgenic mouse model for chronic kidney disease. *EJNMMI Research*, 6, 54.
- Perek, N., Prevot, N., Koumanov, F., Frere, D., Sabido, O., Beauchesne, P., et al. (2000).

Involvement of the glutathione S-conjugate compounds and the MRP protein in Tc-99m-tetrofosmin and Tc-99m-sestamibi uptake in glioma cell lines. *Nuclear Medicine and Biology*, 27, 299–307.

- Pfeifer, N. D., Goss, S. L., Swift, B., Ghibellini, G., Ivanovic, M., Heizer, W. D., et al. (2013). Effect of Ritonavir on (99m)Technetium-Mebrofenin Disposition in Humans: A Semi-PBPK Modeling and In Vitro Approach to Predict Transporter-Mediated DDIs. *CPT: Pharmacometrics & Systems Pharmacology*, 2, e20.
- Pike, V. W. (2009). PET radiotracers: crossing the blood-brain barrier and surviving metabolism. *Trends in Pharmacological Sciences*, 30, 431–440.
- Piwnica-Worms, D., Chiu, M. L., Budding, M., Kronauge, J. F., Kramer, R. A., & Croop, J. M. (1993). Functional imaging of multidrug-resistant P-glycoprotein with an organotechnetium complex. *Cancer Research*, 53, 977–984.
- Planchamp, C., Hadengue, A., Stieger, B., Bourquin, J., Vonlaufen, A., Frossard, J.-L., et al. (2007). Function of both sinusoidal and canalicular transporters controls the concentration of organic anions within hepatocytes. *Molecular Pharmacology*, 71, 1089–1097.
- Pottier, G., Marie, S., Goutal, S., Auvity, S., Peyronneau, M.-A., Stute, S., et al. (2016). Imaging the Impact of the P-Glycoprotein (ABCB1) Function on the Brain Kinetics of Metoclopramide. *Journal of Nuclear Medicine*, 57, 309–314.
- Prasad, B., Lai, Y., Lin, Y., & Unadkat, J. D. (2013). Interindividual variability in the hepatic expression of the human breast cancer resistance protein (BCRP/ABCG2): effect of age, sex, and genotype. *Journal of Pharmaceutical Sciences*, 102, 787–793.
- Raaphorst, R. M., Windhorst, A. D., Elsinga, P. H., Colabufo, N. A., Lammertsma, A. A., & Luurtsema, G. (2015). Radiopharmaceuticals for assessing ABC transporters at the blood-brain barrier. *Clinical Pharmacology and Therapeutics*, 97, 362–371.
- Reichart, D., Reichenspurner, H., & Barten, M. J. (2017). Renal Protection Strategies after Heart Transplantation. *Clinical Transplantation*. doi:10.1111/ctr.13157
- Rizk ML, Zou L, Savic RM, & Dooley KE. (2017). Importance of Drug Pharmacokinetics at the Site of Action. *Clinical and Translational Science*, 10, 133–142.
- Römermann, K., Wanek, T., Bankstahl, M., Bankstahl, J. P., Fedrowitz, M., Müller, M., et al. (2013). (R)-[(11)C]verapamil is selectively transported by murine and human P-glycoprotein at the blood-brain barrier, and not by MRP1 and BCRP. *Nuclear Medicine and Biology*, 40, 873–878.
- Ruparelia, P., Cheow, H. K., Evans, J. W., Banney, L., Shankar, S., Szczepura, K. R., et al. (2008). Pulmonary elimination rate of inhaled 99mTc-sestamibi radioaerosol is delayed in healthy cigarette smokers. *British Journal of Clinical Pharmacology*, 65, 611–614.
- Saito, K., Ledsam, J., Sourbron, S., Otaka, J., Araki, Y., Akata, S., et al. (2013). Assessing liver function using dynamic Gd-EOB-DTPA-enhanced MRI with a standard 5-phase imaging protocol. *Journal of Magnetic Resonance Imaging*, 37, 1109–1114.
- Sakamoto, A., Matsumaru, T., Yamamura, N., Uchida, Y., Tachikawa, M., Ohtsuki, S., et al. (2013). Quantitative expression of human drug transporter proteins in lung tissues: Analysis of regional, gender, and interindividual differences by liquid chromatography–tandem mass

- spectrometry. *Journal of Pharmaceutical Sciences*, 102, 3395–3406.
- Sasongko, L., Link, J. M., Muzi, M., Mankoff, D. A., Yang, X., Collier, A. C., et al. (2005). Imaging P-glycoprotein transport activity at the human blood-brain barrier with positron emission tomography. *Clinical Pharmacology and Therapeutics*, 77, 503–514.
- Schacht, A. C., Sørensen, M., Munk, O. L., & Frisch, K. (2016). Radiosynthesis of N-¹¹C-Methyl-Taurine-Conjugated Bile Acids and Biodistribution Studies in Pigs by PET/CT. *Journal of Nuclear Medicine*, 57, 628–633.
- Schmassmann, A., Angellotti, M. A., Ton-Nu, H. T., Schteingart, C. D., Marcus, S. N., Rossi, S. S., et al. (1990). Transport, metabolism, and effect of chronic feeding of cholylsarcosine, a conjugated bile acid resistant to deconjugation and dehydroxylation. *Gastroenterology*, 98, 163–174.
- Seithel, A., Eberl, S., Singer, K., Auge, D., Heinkele, G., Wolf, N. B., et al. (2007). The influence of macrolide antibiotics on the uptake of organic anions and drugs mediated by OATP1B1 and OATP1B3. *Drug Metabolism and Disposition*, 35, 779–786.
- Shawahna, R., Uchida, Y., Declèves, X., Ohtsuki, S., Yousif, S., Dauchy, S., et al. (2011). Transcriptomic and quantitative proteomic analysis of transporters and drug metabolizing enzymes in freshly isolated human brain microvessels. *Molecular Pharmaceutics*, 8, 1332–1341.
- Shikano, N., Kanai, Y., Kawai, K., Ishikawa, N., & Endou, H. (2004). Transport of ^{99m}Tc-MAG3 via rat renal organic anion transporter 1. *Journal of Nuclear Medicine*, 45, 80–85.
- Shin, J.-W., Chu, K., Shin, S. A., Jung, K.-H., Lee, S.-T., Lee, Y.-S., et al. (2016). Clinical Applications of Simultaneous PET/MR Imaging Using (R)-[¹¹C]-Verapamil with Cyclosporin A: Preliminary Results on a Surrogate Marker of Drug-Resistant Epilepsy. *AJNR. American Journal of Neuroradiology*, 37, 600–606.
- Shingaki, T., Hume, W. E., Takashima, T., Katayama, Y., Okauchi, T., Hayashinaka, E., et al. (2015). Quantitative Evaluation of mMate1 Function Based on Minimally Invasive Measurement of Tissue Concentration Using PET with [(11)C]Metformin in Mouse. *Pharmaceutical Research*, 32, 2538–2547.
- Shingaki, T., Takashima, T., Ijuin, R., Zhang, X., Onoue, T., Katayama, Y., et al. (2013). Evaluation of Oatp and Mrp2 Activities in Hepatobiliary Excretion Using Newly Developed Positron Emission Tomography Tracer [¹¹C]Dehydropravastatin in Rats. *Journal of Pharmacology and Experimental Therapeutics*, 347, 193–202.
- Sinharay, S., & Pagel, M. D. (2016). Advances in Magnetic Resonance Imaging Contrast Agents for Biomarker Detection. *Annual Review of Analytical Chemistry*, 9, 95–115.
- Sivapackiam, J., Harpstrite, S. E., Prior, J. L., Mattingly, S., & Sharma, V. (2016). (67/68)Ga⁶⁷Galmydar: A metalloprobe for monitoring breast cancer resistance protein (BCRP)-mediated functional transport activity. *Nuclear Medicine and Biology*, 43, 191–197.
- Sørensen, M., Munk, O. L., Ørntoft, N. W., Frisch, K., Andersen, K. J., Mortensen, F. V., et al. (2016). Hepatobiliary Secretion Kinetics of Conjugated Bile Acids Measured in Pigs by ¹¹C-Cholylsarcosine PET. *Journal of Nuclear Medicine*, 57, 961–966.
- Sourbron, S. P., & Buckley, D. L. (2013). Classic models for dynamic contrast-enhanced MRI.

NMR in Biomedicine, 26, 1004–1027.

- Sourbron, S., Sommer, W. H., Reiser, M. F., & Zech, C. J. (2012). Combined quantification of liver perfusion and function with dynamic gadoxetic acid-enhanced MR imaging. *Radiology*, 263, 874–883.
- Srere, P. A., & Ovadi, J. (1990). Enzyme-enzyme interactions and their metabolic role. *FEBS Letters*, 268, 360–364.
- Stieger, B. (2011). The role of the sodium-taurocholate cotransporting polypeptide (NTCP) and of the bile salt export pump (BSEP) in physiology and pathophysiology of bile formation. *Handbook of Experimental Pharmacology*, 205–259.
- Stieger, B., Fattinger, K., Madon, J., Kullak-Ublick, G. A., & Meier, P. J. (2000). Drug- and estrogen-induced cholestasis through inhibition of the hepatocellular bile salt export pump (Bsep) of rat liver. *Gastroenterology*, 118, 422–430.
- Stieger, B., & Gao, B. (2015). Drug transporters in the central nervous system. *Clinical Pharmacokinetics*, 54, 225–242.
- Stieger, B., Heger, M., de Graaf, W., Paumgartner, G., & van Gulik, T. (2012). The emerging role of transport systems in liver function tests. *European Journal of Pharmacology*, 675, 1–5.
- Stieger, B., Unadkat, J. D., Prasad, B., Langer, O., & Gali, H. (2014). Role of (drug) transporters in imaging in health and disease. *Drug Metabolism and Disposition*, 42, 2007–2015.
- Sundelin, E., Gormsen, L. C., Jensen, J. B., Vendelbo, M. H., Jakobsen, S., Munk, O. L., et al. (2017). Genetic Polymorphisms in Organic Cation Transporter 1 Attenuates Hepatic Metformin Exposure in Humans. *Clinical Pharmacology and Therapeutics*, 102, 841–848.
- Suzuki, H., & Sugiyama, Y. (2000). Transport of drugs across the hepatic sinusoidal membrane: sinusoidal drug influx and efflux in the liver. *Seminars in Liver Disease*, 20, 251–263.
- Syvänen, S., Lindhe, O., Palner, M., Kornum, B. R., Rahman, O., Långström, B., et al. (2009). Species differences in blood-brain barrier transport of three positron emission tomography radioligands with emphasis on P-glycoprotein transport. *Drug Metabolism and Disposition*, 37, 635–643.
- Syvänen, S., Xie, R., Sahin, S., & Hammarlund-Udenaes, M. (2006). Pharmacokinetic consequences of active drug efflux at the blood-brain barrier. *Pharmaceutical Research*, 23, 705–717.
- Szabo, Z., Alachkar, N., Xia, J., Mathews, W. B., & Rabb, H. (2011). Molecular imaging of the kidneys. *Seminars in Nuclear Medicine*, 41, 20–28.
- Szakács, G., Paterson, J. K., Ludwig, J. A., Booth-Genthe, C., & Gottesman, M. M. (2006). Targeting multidrug resistance in cancer. *Nature Reviews. Drug Discovery*, 5, 219–234.
- Takano, A., Kusuhara, H., Suhara, T., Ieiri, I., Morimoto, T., Lee, Y.-J., et al. (2006). Evaluation of in vivo P-glycoprotein function at the blood-brain barrier among MDR1 gene polymorphisms by using ¹¹C-verapamil. *Journal of Nuclear Medicine*, 47, 1427–1433.
- Takano, H., Ito, S., Zhang, X., Ito, H., Zhang, M.-R., Suzuki, H., et al. (2017). Possible Role of Organic Cation Transporters in the Distribution of [¹¹C]Sulpiride, a Dopamine D2 Receptor

Antagonist. *Journal of Pharmaceutical Sciences*, 106, 2558–2565.

- Takashima, T., Kitamura, S., Wada, Y., Tanaka, M., Shigihara, Y., Ishii, H., et al. (2012). PET imaging-based evaluation of hepatobiliary transport in humans with (15R)-11C-TIC-Me. *Journal of Nuclear Medicine*, 53, 741–748.
- Takashima, T., Wu, C., Takashima-Hirano, M., Katayama, Y., Wada, Y., Suzuki, M., et al. (2013). Evaluation of breast cancer resistance protein function in hepatobiliary and renal excretion using PET with 11C-SC-62807. *Journal of Nuclear Medicine*, 54, 267–276.
- Taki, J., Sumiya, H., Asada, N., Ueda, Y., Tsuchiya, H., & Tonami, N. (1998). Assessment of P-glycoprotein in patients with malignant bone and soft-tissue tumors using technetium-99m-MIBI scintigraphy. *Journal of Nuclear Medicine*, 39, 1179–1184.
- Taskar, K. S., Mariappan, T. T., Kurawattimath, V., Singh Gautam, S., Radhakrishna Mullapudi, T. V., Sridhar, S. K., et al. (2017). Unmasking the Role of Uptake Transporters for Digoxin Uptake Across the Barriers of the Central Nervous System in Rat. *Journal of Central Nervous System Disease*, 9, 1179573517693596.
- Taylor, A. T. (2014). Radionuclides in Nephrourology, Part 1: Radiopharmaceuticals, Quality Control, and Quantitative Indices. *Journal of Nuclear Medicine*, 55, 608–615.
- Testa, A., Zanda, M., Elmore, C. S., & Sharma, P. (2015). PET Tracers To Study Clinically Relevant Hepatic Transporters. *Molecular Pharmaceutics*, 12, 2203–2216.
- Thian, Y. L., Riddell, A. M., & Koh, D.-M. (2013). Liver-specific agents for contrast-enhanced MRI: role in oncological imaging. *Cancer Imaging*, 13, 567–579.
- Toornvliet, R., van Berckel, B. N. M., Luurtsema, G., Lubberink, M., Geldof, A. A., Bosch, T. M., et al. (2006). Effect of age on functional P-glycoprotein in the blood-brain barrier measured by use of (R)-[(11)C]verapamil and positron emission tomography. *Clinical Pharmacology and Therapeutics*, 79, 540–548.
- Tournier, N., André, P., Blondeel, S., Rizzo-Padoin, N., du Moulinet d'Hardemarre, A., Declèves, X., et al. (2009). Ibogaine labeling with 99mTc-tricarbonyl: synthesis and transport at the mouse blood-brain barrier. *Journal of Pharmaceutical Sciences*, 98, 4650–4660.
- Tournier, N., Cisternino, S., Peyronneau, M.-A., Goutal, S., Dolle, F., Scherrmann, J.-M., et al. (2012). Discrepancies in the P-glycoprotein-mediated transport of (18)F-MPPF: a pharmacokinetic study in mice and non-human primates. *Pharmaceutical Research*, 29, 2468–2476.
- Tournier, N., Goutal, S., Auvity, S., Traxl, A., Mairinger, S., Wanek, T., et al. (2017). Strategies to Inhibit ABCB1- and ABCG2-Mediated Efflux Transport of Erlotinib at the Blood-Brain Barrier: A PET Study on Nonhuman Primates. *Journal of Nuclear Medicine*, 58, 117–122.
- Tournier, N., Saba, W., Cisternino, S., Peyronneau, M.-A., Damont, A., Goutal, S., et al. (2013). Effects of selected OATP and/or ABC transporter inhibitors on the brain and whole-body distribution of glyburide. *The AAPS Journal*, 15, 1082–1090.
- Tournier, N., Valette, H., Peyronneau, M.-A., Saba, W., Goutal, S., Kuhnast, B., et al. (2011). Transport of selected PET radiotracers by human P-glycoprotein (ABCB1) and breast cancer resistance protein (ABCG2): an in vitro screening. *Journal of Nuclear Medicine*, 52, 415–423.

- Traxl, A., Komposch, K., Glitzner, E., Wanek, T., Mairinger, S., Langer, O., et al. (2017). Hepatocyte-Specific Deletion of EGFR in Mice Reduces Hepatic Abcg2 Transport Activity Measured by [(11)C]erlotinib and Positron Emission Tomography. *Drug Metabolism and Disposition*, 45, 1093–1100.
- Traxl, A., Wanek, T., Mairinger, S., Stanek, J., Filip, T., Sauberer, M., et al. (2015). Breast Cancer Resistance Protein and P-Glycoprotein Influence In Vivo Disposition of 11C-Erlotinib. *Journal of Nuclear Medicine*, 56, 1930–1936.
- Tsuboyama, T., Onishi, H., Kim, T., Akita, H., Hori, M., Tatsumi, M., et al. (2010). Hepatocellular carcinoma: hepatocyte-selective enhancement at gadoxetic acid-enhanced MR imaging--correlation with expression of sinusoidal and canalicular transporters and bile accumulation. *Radiology*, 255, 824–833.
- Uchida, Y., Ohtsuki, S., Katsukura, Y., Ikeda, C., Suzuki, T., Kamiie, J., et al. (2011). Quantitative targeted absolute proteomics of human blood-brain barrier transporters and receptors. *Journal of Neurochemistry*, 117, 333–345.
- Vallabhajosula, S., Solnes, L., & Vallabhajosula, B. (2011). A broad overview of positron emission tomography radiopharmaceuticals and clinical applications: what is new? *Seminars in Nuclear Medicine*, 41, 246–264.
- Vallon, V., Rieg, T., Ahn, S. Y., Wu, W., Eraly, S. A., & Nigam, S. K. (2008). Overlapping in vitro and in vivo specificities of the organic anion transporters OAT1 and OAT3 for loop and thiazide diuretics. *American Journal of Physiology. Renal Physiology*, 294, F867–873.
- van Assema, D. M. E., Lubberink, M., Bauer, M., van der Flier, W. M., Schuit, R. C., Windhorst, A. D., et al. (2012a). Blood-brain barrier P-glycoprotein function in Alzheimer's disease. *Brain*, 135, 181–189.
- van Assema, D. M. E., Lubberink, M., Boellaard, R., Schuit, R. C., Windhorst, A. D., Scheltens, P., et al. (2012b). P-glycoprotein function at the blood-brain barrier: effects of age and gender. *Molecular Imaging and Biology*, 14, 771–776.
- van Assema, D. M. E., Lubberink, M., Rizzu, P., van Swieten, J. C., Schuit, R. C., Eriksson, J., et al. (2012c). Blood-brain barrier P-glycoprotein function in healthy subjects and Alzheimer's disease patients: effect of polymorphisms in the ABCB1 gene. *EJNMMI Research*, 2, 57.
- Van Beers, B. E., Pastor, C. M., & Hussain, H. K. (2012). Primovist, Eovist: what to expect? *Journal of Hepatology*, 57, 421–429.
- van der Veldt, A. A. M., Lubberink, M., Mathijssen, R. H. J., Loos, W. J., Herder, G. J. M., Greuter, H. N., et al. (2013a). Toward prediction of efficacy of chemotherapy: a proof of concept study in lung cancer patients using [¹¹C]docetaxel and positron emission tomography. *Clinical Cancer Research*, 19, 4163–4173.
- van der Veldt, A. A. M., Smit, E. F., & Lammertsma, A. A. (2013b). Positron Emission Tomography as a Method for Measuring Drug Delivery to Tumors in vivo: The Example of [(11)C]docetaxel. *Frontiers in Oncology*, 3, 208.
- van Montfoort, J. E., Hagenbuch, B., Groothuis, G. M. M., Koepsell, H., Meier, P. J., & Meijer, D. K. F. (2003). Drug uptake systems in liver and kidney. *Current Drug Metabolism*, 4, 185–211.
- van Montfoort, J. E., Stieger, B., Meijer, D. K., Weinmann, H. J., Meier, P. J., & Fattinger, K. E.

- (1999). Hepatic uptake of the magnetic resonance imaging contrast agent gadoxetate by the organic anion transporting polypeptide Oatp1. *The Journal of Pharmacology and Experimental Therapeutics*, 290, 153–157.
- Varma, M. V., & El-Kattan, A. F. (2016). Transporter-Enzyme Interplay: Deconvoluting Effects of Hepatic Transporters and Enzymes on Drug Disposition Using Static and Dynamic Mechanistic Models. *Journal of Clinical Pharmacology*, 56 Suppl 7, S99–S109.
- Varma, M. V., Pang, K. S., Isoherranen, N., & Zhao, P. (2015). Dealing with the complex drug-drug interactions: towards mechanistic models. *Biopharmaceutics & Drug Disposition*, 36, 71–92.
- Verheijen, R. B., Yaqub, M. M., Sawicki, E., van Tellingen, O., Lammertsma, A. A., Nuijen, B., et al. (2017). Molecular Imaging of ABCB1/ABCG2 Inhibition at the Human Blood Brain Barrier using Elacridar and 11C-Erlotinib PET. *Journal of Nuclear Medicine*. doi:10.2967/jnumed.117.195800
- Vilgrain, V., Van Beers, B. E., & Pastor, C. M. (2016). Insights into the diagnosis of hepatocellular carcinomas with hepatobiliary MRI. *Journal of Hepatology*, 64, 708–716.
- Visentin, M., van Rosmalen, B. V., Hiller, C., Bieze, M., Hofstetter, L., Verheij, J., et al. (2017). Impact of Organic Cation Transporters (OCT-SLC22A) on Differential Diagnosis of Intrahepatic Lesions. *Drug Metabolism and Disposition*, 45, 166–173.
- Wagner, C. C., Bauer, M., Karch, R., Feurstein, T., Kopp, S., Chiba, P., et al. (2009). A pilot study to assess the efficacy of tariquidar to inhibit P-glycoprotein at the human blood-brain barrier with (R)-11C-verapamil and PET. *Journal of Nuclear Medicine*, 50, 1954–1961.
- Wanek, T., Halilbasic, E., Visentin, M., Mairinger, S., Römermann, K., Stieger, B., et al. (2016). Influence of 24-Nor-Ursodeoxycholic Acid on Hepatic Disposition of [(18F)]Ciprofloxacin, a Positron Emission Tomography Study in Mice. *Journal of Pharmaceutical Sciences*, 105, 106–112.
- Wanek, T., Kuntner, C., Bankstahl, J. P., Mairinger, S., Bankstahl, M., Stanek, J., et al. (2012). A novel PET protocol for visualization of breast cancer resistance protein function at the blood-brain barrier. *Journal of Cerebral Blood Flow and Metabolism*, 32, 2002–2011.
- Wanek, T., Mairinger, S., & Langer, O. (2013). Radioligands targeting P-glycoprotein and other drug efflux proteins at the blood-brain barrier. *Journal of Labelled Compounds & Radiopharmaceuticals*, 56, 68–77.
- Wanek, T., Römermann, K., Mairinger, S., Stanek, J., Sauberer, M., Filip, T., et al. (2015). Factors Governing P-Glycoprotein-Mediated Drug-Drug Interactions at the Blood-Brain Barrier Measured with Positron Emission Tomography. *Molecular Pharmaceutics*, 12, 3214–3225.
- Willmann, J. K., van Bruggen, N., Dinkelborg, L. M., & Gambhir, S. S. (2008). Molecular imaging in drug development. *Nature Reviews. Drug Discovery*, 7, 591–607.
- Wuest, F. (2007). Fluorine-18 labeling of small molecules: the use of 18F-labeled aryl fluorides derived from no-carrier-added [18F]fluoride as labeling precursors. *Ernst Schering Research Foundation Workshop*, 51–78.
- Wulkersdorfer, B., Wanek, T., Bauer, M., Zeitlinger, M., Müller, M., & Langer, O. (2014). Using positron emission tomography to study transporter-mediated drug-drug interactions in tissues.

Clinical Pharmacology and Therapeutics, 96, 206–213.

- Xia, C. Q., Xiao, G., Liu, N., Pimprale, S., Fox, L., Patten, C. J., et al. (2006). Comparison of species differences of P-glycoproteins in beagle dog, rhesus monkey, and human using Atpase activity assays. *Molecular Pharmaceutics*, 3, 78–86.
- Xiao, Y.-D., Paudel, R., Liu, J., Ma, C., Zhang, Z.-S., & Zhou, S.-K. (2016). MRI contrast agents: Classification and application (Review). *International Journal of Molecular Medicine*, 38, 1319–1326.
- Yamasaki, Y., Ieiri, I., Kusuhara, H., Sasaki, T., Kimura, M., Tabuchi, H., et al. (2008). Pharmacogenetic characterization of sulfasalazine disposition based on NAT2 and ABCG2 (BCRP) gene polymorphisms in humans. *Clinical Pharmacology and Therapeutics*, 84, 95–103.
- Yamazaki, M., Neway, W. E., Ohe, T., Chen, I., Rowe, J. F., Hochman, J. H., et al. (2001). In vitro substrate identification studies for p-glycoprotein-mediated transport: species difference and predictability of in vivo results. *The Journal of Pharmacology and Experimental Therapeutics*, 296, 723–735.
- Yin, J., & Wang, J. (2016). Renal drug transporters and their significance in drug–drug interactions. *Acta Pharmaceutica Sinica. B*, 6, 363–373.
- Yoneda, N., Matsui, O., Kitao, A., Kita, R., Kozaka, K., Koda, W., et al. (2013). Hypervascular hepatocellular carcinomas showing hyperintensity on hepatobiliary phase of gadoxetic acid-enhanced magnetic resonance imaging: a possible subtype with mature hepatocyte nature. *Japanese Journal of Radiology*, 31, 480–490.
- Yoneda, N., Matsui, O., Kitao, A., Kozaka, K., Kobayashi, S., Sasaki, M., et al. (2016). Benign Hepatocellular Nodules: Hepatobiliary Phase of Gadoteric Acid-enhanced MR Imaging Based on Molecular Background. *Radiographics*, 36, 2010–2027.
- Zamek-Gliszczynski, M. J., Giacomini, K. M., & Zhang, L. (2017). Emerging Clinical Importance of Hepatic Organic Cation Transporter 1 (OCT1) in Drug Pharmacokinetics, Dynamics, Pharmacogenetic Variability, and Drug Interactions. *Clinical Pharmacology and Therapeutics*. doi:10.1002/cpt.941
- Zheng, H. X., Huang, Y., Frassetto, L. A., & Benet, L. Z. (2009). Elucidating rifampin's inducing and inhibiting effects on glyburide pharmacokinetics and blood glucose in healthy volunteers: unmasking the differential effects of enzyme induction and transporter inhibition for a drug and its primary metabolite. *Clinical Pharmacology and Therapeutics*, 85, 78–85.

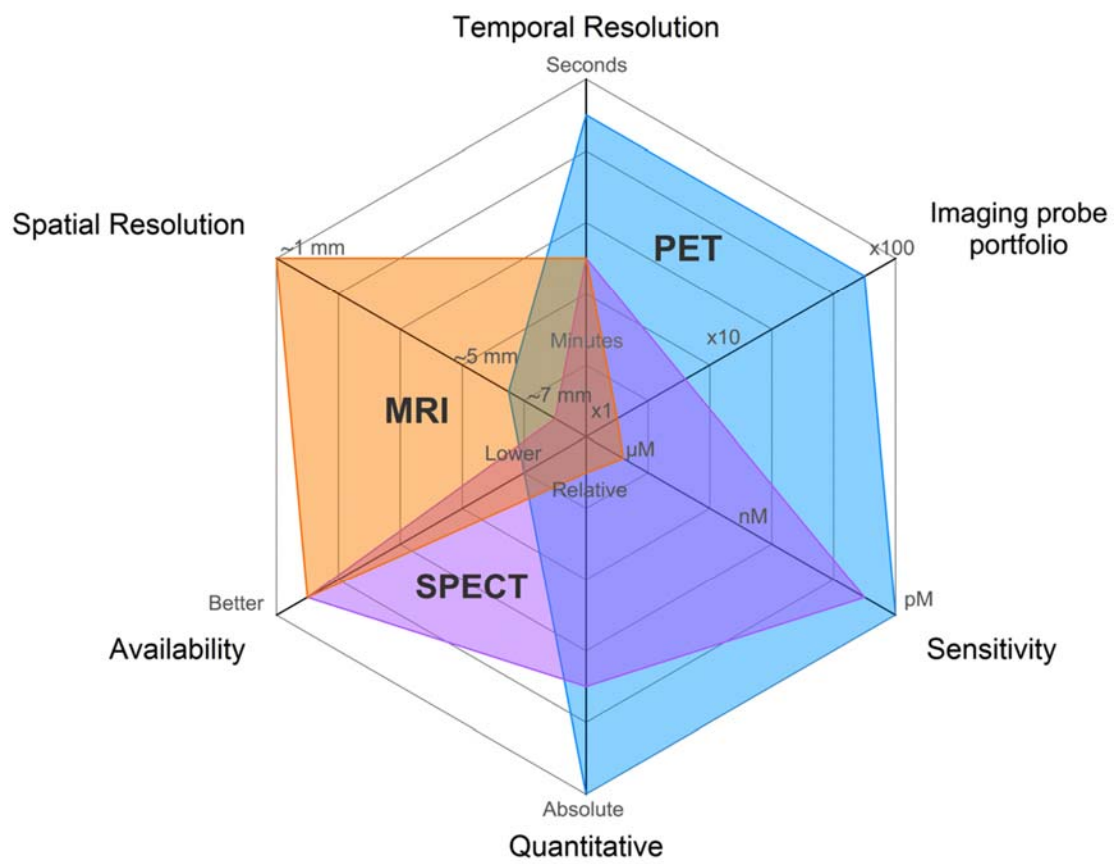


Figure 1

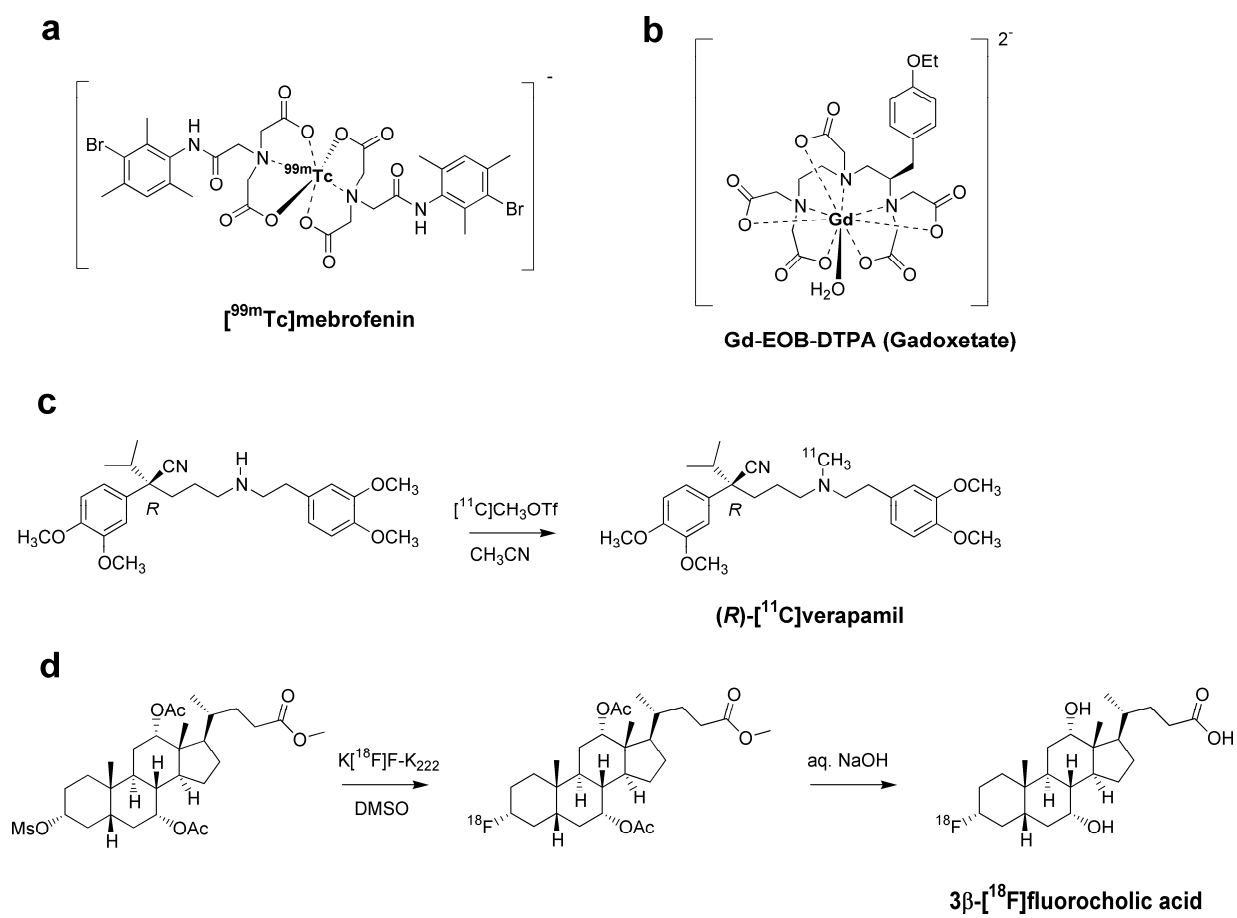


Figure 2

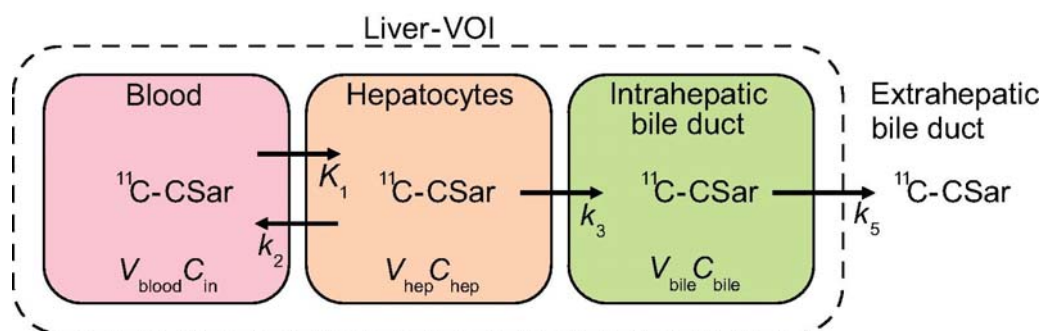


Figure 3

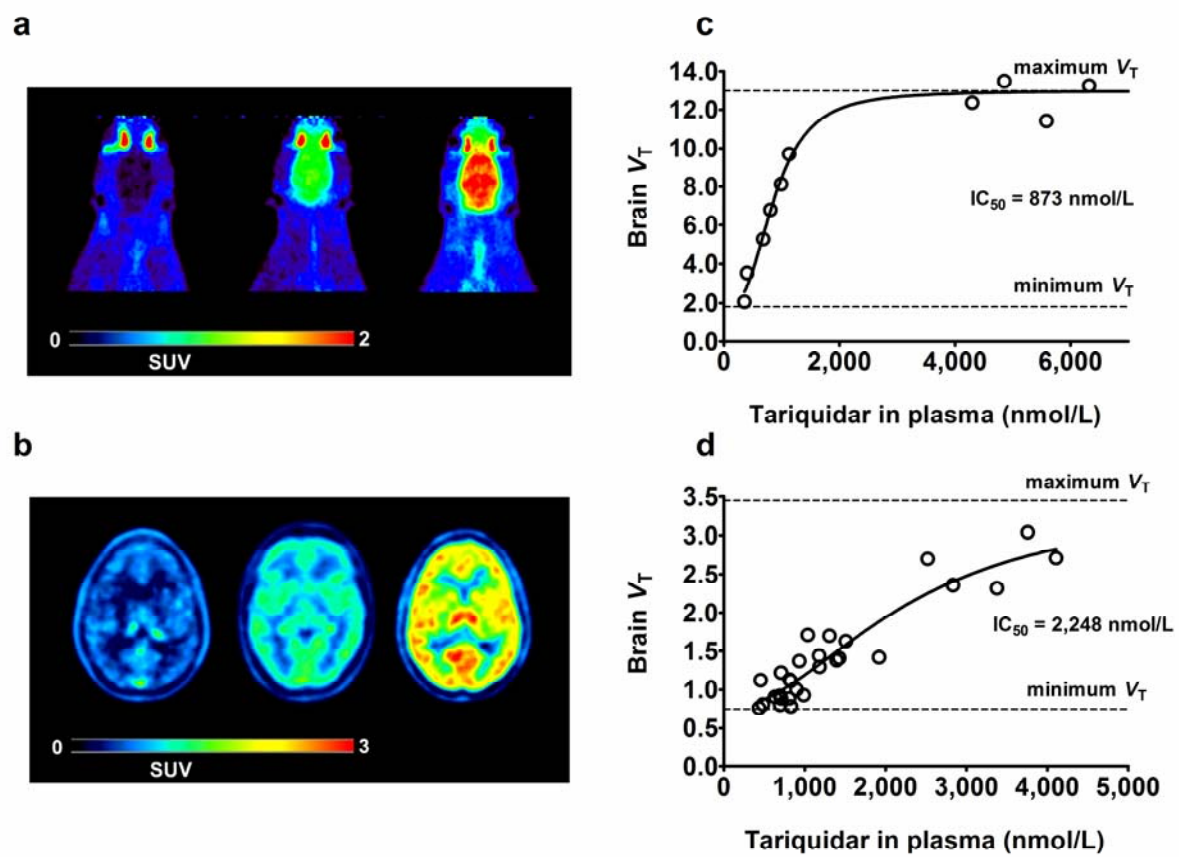


Figure 4

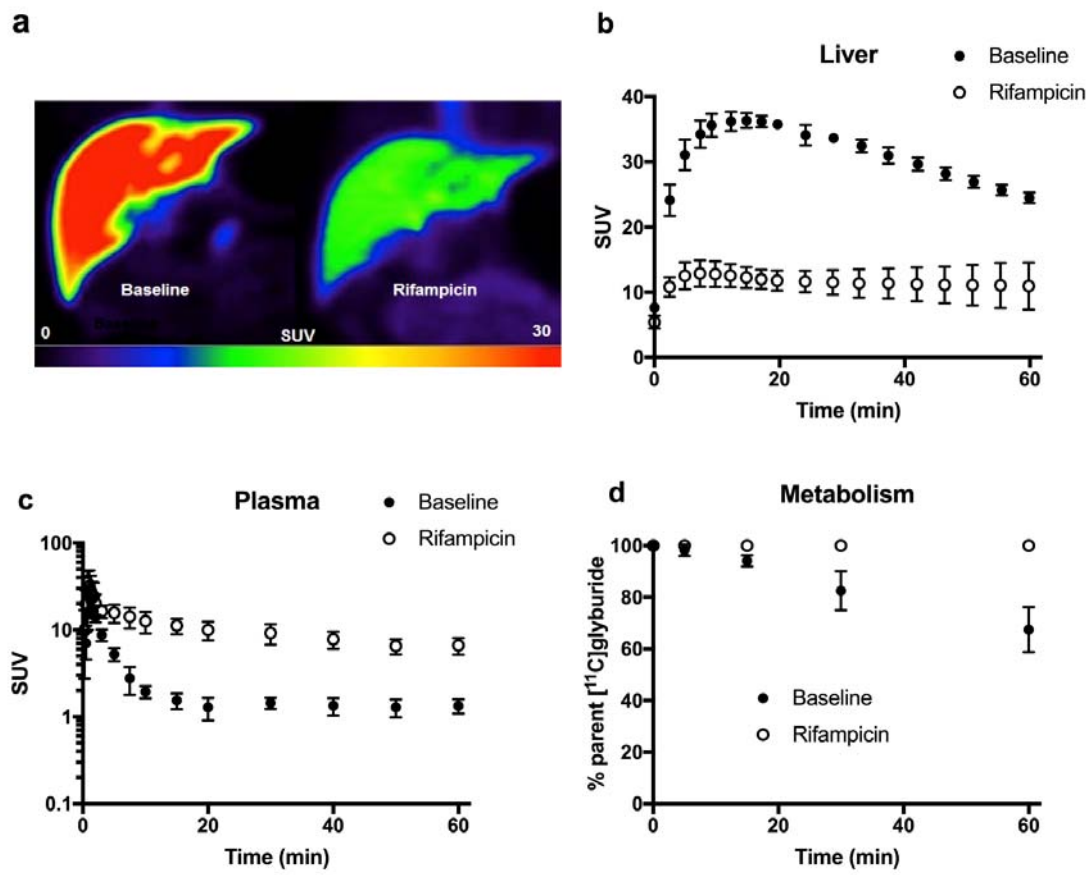


Figure 5

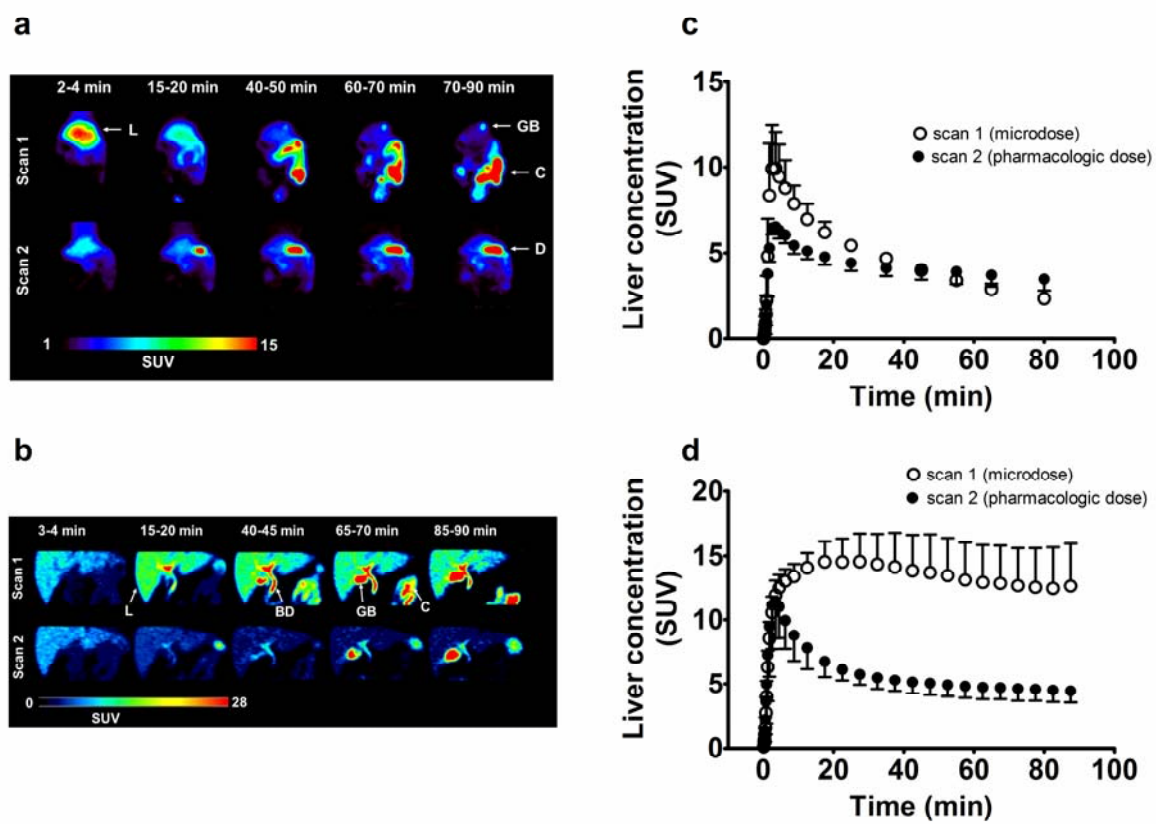


Figure 6